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Supporting Information

The Discovery and Structure-Activity Evaluation of (+)-Floyocidin B and Synthetic Analogs

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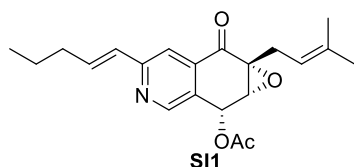
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1. Experimental Procedures

For the avicennone C-floyocidin B hybrids, the synthetic steps are described in details for the series with the same absolute stereochemistry of the epoxide as (+)-floyocidin B (**4**) and the corresponding stereoisomers are listed afterwards.

1.1. Synthetic procedures and compound characterization

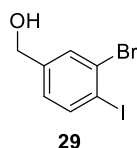
(1*aR*,2*R*,7*aR*)-7*a*-(3-methylbut-2-en-1-yl)-7-oxo-5-((*E*)-pent-1-en-1-yl)-1*a*,2,7,7*a*-tetrahydrooxireno[2,3-*g*]isoquinolin-2-yl acetate (**SI1**):



Alcohol **27** (0.016 g, 0.051 mmol, 1.00 eq.) was dissolved in anhydrous THF (5 mL). PPh₃ (0.040 g, 0.15 mmol, 3.00 eq.) and HOAc (0.018 mL, 0.31 mmol, 6.00 eq.) were added. Then DIAD (0.030 mL, 0.15 mmol, 3.00 eq.) was added at 0 °C. The reaction solution was stirred at room temperature for 1 h 10 min until TLC showed complete conversion of the starting material. The reaction mixture was loaded on silica and was then purified *via* flash column chromatography (0–40% ethyl acetate in *n*-heptane) to give **SI1** (0.015 g; 0.043 mmol; 84%) as colorless oil.

¹H-NMR (CDCl₃, 400 MHz): δ = 8.54 (s, 1H, N-CH), 7.64 (s, 1H, pentenyl-C_q-CH), 6.82 (dt, 1H, *J* = 15.7, 7.0 Hz, CH₂-CH=CH), 6.53 (dt, 1H, *J* = 15.7, 1.5 Hz, CH₂-CH=CH), 6.36 (s, br, 1H, CH-OAc), 5.06 (m, 1H, Me₂C=CH), 3.89 (d, 1H, *J* = 2.0 Hz, epoxy-CH), 2.91 (dd, 1H, *J* = 15.5, 8.0 Hz, Me₂C=CH-CH₂), 2.63 (dd, 1H, *J* = 15.5, 7.0 Hz, Me₂C=CH-CH₂), 2.32 (s, 3H, CO-CH₃), 2.26 (m, 2H, CH₂-CH=CH), 1.73 (s, 3H, *E*-CH₃), 1.68 (s, 3H, *Z*-CH₃), 1.53 (m, 2H, CH₃-CH₂), 0.96 (t, 3H, *J* = 7.3 Hz, CH₃-CH₂); **¹³C-NMR** (CDCl₃, 100 MHz): δ = 193.5 (C=O), 170.9 (CO-CH₃), 157.4 (N-C_q), 148.5 (N-CH), 138.2 (CH₂-CH=CH), 137.2 (C_qMe₂), 136.2 (Ar-C_q-CO), 129.1 (CH₂-CH=CH), 126.8 (N-CH-C_q), 116.6 (pentenyl-C_q-CH), 115.7 (CH=CMe₂), 66.3 (CH-OAc), 61.8 (epoxy-C_q), 56.5 (epoxy-CH), 35.1 (CH₂-CH=CH), 26.2 (Me₂C=CH-CH₂), 26.0 (*E*-CH₃), 22.2 (CH₃-CH₂), 21.1 (CO-CH₃), 18.2 (*Z*-CH₃), 13.9 (CH₃-CH₂); **HRMS (ESI)** *m/z* calcd. for C₂₁H₂₆NO₄: 356.1856 (M+H)⁺; found: 356.1853 (M+H)⁺; **R_f** (*n*-heptane/ethyl acetate 2:1): 0.37; **Specific rotation** [α]_D^{25.6} = +193.8 (*c* = 0.61; CHCl₃).

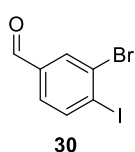
(3-Bromo-4-iodophenyl)methanol (**29**):



3-Bromo-4-iodobenzoic acid (4.98 g, 15.2 mmol, 1.00 eq.) was suspended in anhydrous DCM (10 mL) and (COCl)₂ (1.90 mL, 22.2 mmol, 1.46 eq.) as well as two drops of anhydrous DMF were added. The resulting mixture was stirred at room temperature for 1 h 45 min and was then concentrated *in vacuo*. The residue was dissolved in anhydrous THF (10 mL) and LiBH₄ (0.334 g, 15.3 mmol, 1.00 eq.) was added. After 3 h 30 min stirring at room temperature, saturated aqueous NH₄Cl (20 mL) was added. The mixture was extracted with ethyl acetate (2 x 100 mL) and the combined organic layers were washed with saturated aqueous NaCl (100 mL), dried over MgSO₄, filtered, and concentrated under reduced pressure. The crude product was purified by flash column chromatography (*n*-heptane/ethyl acetate 10:1 to 2:1) to give **29** (4.42 g, 14.1 mmol, 93% over two steps) as colorless solid.

¹H-NMR (CDCl₃, 400 MHz): δ = 7.82 (d, 1H, *J* = 8.1 Hz, IC_q-CH), 7.63 (d, 1H, *J* = 1.9 Hz, BrC_q-CH), 6.98 (dd, 1H, *J* = 8.1, 1.9 Hz, CH-CH-C_q-CH₂), 4.62 (s, 2H, CH₂), 1.88 (s, br, 1H, OH); **¹³C-NMR** (CDCl₃, 100 MHz): δ = 142.9 (BrC_q), 140.4 (IC_q-CH), 131.1 (BrC_q-CH), 130.0 (CH₂-C_q), 126.9 (CH-CH-C_q-CH₂), 99.7 (IC_q), 64.0 (CH₂); **HRMS (ESI)** *m/z* calcd. for C₇H₅BrI: 294.8614 (M-H₂O+H)⁺; found: 294.8611 (M-H₂O+H)⁺; **R_f** (*n*-heptane/ethyl acetate 4:1): 0.17.

3-Bromo-4-iodobenzaldehyde (30):

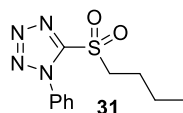


The reaction was carried out in moisture-free glassware under argon atmosphere. To a solution of $(\text{COCl})_2$ (1.70 mL, 22.0 mmol, 1.80 eq.) in anhydrous DCM (90 mL), anhydrous DMSO (2.70 mL, 38.0 mmol, 3.20 eq.) was added carefully at $-78\text{ }^\circ\text{C}$. After stirring for 15 min, alcohol **29** (3.73 g, 11.9 mmol, 1.00 eq.) dissolved in anhydrous DCM (72 mL) was added. The reaction mixture was stirred for 1 h 30 min at $-78\text{ }^\circ\text{C}$.

Then DIPEA (10.4 mL, 59.7 mmol, 5.01 eq.) was added and the solution was stirred for 30 min at $-78\text{ }^\circ\text{C}$ and for further 30 min without cooling bath. A mixture of toluene/saturated aqueous NH_4Cl (3:1, 240 mL) was added to the reaction mixture. The aqueous layer was separated and the organic layer was washed again with saturated aqueous NH_4Cl (3 x 80 mL), with saturated aqueous NaHCO_3 (80 mL), and with saturated aqueous NaCl (80 mL). The organic layer was dried over MgSO_4 , filtered, and concentrated under reduced pressure to yield aldehyde **30** (11.9 mmol, quant.) as beige solid which was used in the next stage without further purification. For full characterization a small portion of the crude product was purified by flash column chromatography (0–5% ethyl acetate in *n*-heptane).

$^1\text{H-NMR}$ (CDCl_3 , 400 MHz): δ = 9.91 (s, 1H, CHO), 8.10–8.03 (m, 2H, $\text{IC}_q\text{-CH}$ and $\text{BrC}_q\text{-CH}$), 7.47 (dd, 1H, J = 8.1, 1.9 Hz, CH-CH- $\text{C}_q\text{-CHO}$); **$^{13}\text{C-NMR}$** (CDCl_3 , 100 MHz): δ = 190.2 (CHO), 141.3 ($\text{IC}_q\text{-CH}$), 137.5 (Ar- C_q), 133.3 ($\text{BrC}_q\text{-CH}$), 131.2 (Ar- C_q), 128.5 (CH-CH- $\text{C}_q\text{-CH}_2$), 109.9 (IC_q); **HRMS (ESI)** m/z calcd. for $\text{C}_7\text{H}_5\text{BrIO}$: 310.8563 ($\text{M}+\text{H}$)⁺; found: 310.8566 ($\text{M}+\text{H}$)⁺; **R_f** (*n*-heptane/ethyl acetate 4:1): 0.50.

5-(Butylsulfonyl)-1-phenyl-1H-tetrazole (31):



1-Phenyl-1H-tetrazol-5-thiol (5.00 g, 28.1 mmol, 1.00 eq.) was dissolved in DMF (15 mL). 1-Bromobutane (3.00 mL, 28.1 mmol, 1.00 eq.) and KOH (2.46 g, 43.9 mmol, 1.50 eq.) were added. After stirring at room temperature for 2 h, the TLC showed some remaining starting material, so a second portion of 1-bromobutane (0.600 mL, 5.60 mmol, 0.20 eq.) was added. The reaction mixture

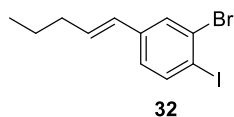
was stirred at room temperature for further 3 h, until the TLC indicated a complete conversion. H_2O (50 mL) was added and the mixture was extracted twice with ethyl acetate (150 mL and 100 mL). The combined organic layers were washed with saturated aqueous NaCl (50 mL), dried over MgSO_4 , filtered, and concentrated under reduced pressure.

The residue was dissolved in DCM (100 mL) and *m*-CPBA ($\geq 70\%$, 21.8 g, 88.3 mmol, 3.04 eq.) was added carefully at $0\text{ }^\circ\text{C}$. The suspension was allowed to stir overnight at room temperature and then saturated aqueous $\text{Na}_2\text{S}_2\text{O}_3\text{-Lsg}$. (60 mL) was added. The mixture was extracted with Et_2O (2 x 300 mL) and the combined organic layers were washed with 1 M aqueous NaOH (2 x 100 mL) and saturated aqueous NaCl (150 mL), dried over MgSO_4 , filtered, and concentrated under reduced pressure to yield **31** (6.92 g, 26.0 mmol, 93% over two steps) as slightly yellow oil, which was used in the next step without further purification.

$^1\text{H-NMR}$ (CDCl_3 , 400 MHz): δ = 7.75–7.55 (m, 5H, Ar- H), 3.79–3.67 (m, 2H, $\text{SO}_2\text{-CH}_2$), 1.99–1.88 (m, 2H, $\text{SO}_2\text{-CH}_2\text{-CH}_2$), 1.54 (m, 2H, $\text{CH}_2\text{-CH}_3$), 0.98 (t, 3H, J = 7.4 Hz, CH_3); **$^{13}\text{C-NMR}$** (CDCl_3 , 100 MHz): δ = 153.6 ($\text{SO}_2\text{-C}_q$), 133.2 (Ar- C_q), 131.6 (Ar-CH), 129.8 (Ar-CH), 125.2 (Ar-CH), 55.9 ($\text{SO}_2\text{-CH}_2$), 24.0 ($\text{SO}_2\text{-CH}_2\text{-CH}_2$), 21.6 ($\text{CH}_2\text{-CH}_3$), 13.5 (CH_3); **HRMS (ESI)** m/z calcd. for $\text{C}_{11}\text{H}_{15}\text{N}_4\text{O}_2\text{S}$: 267.0910 ($\text{M}+\text{H}$)⁺; found: 267.0910 ($\text{M}+\text{H}$)⁺; **R_f** (*n*-heptane/ethyl acetate 4:1): 0.38.

(E)-2-Bromo-1-iodo-4-(pent-1-en-1-yl)benzene (32):

The reaction was carried out in moisture-free glassware under argon atmosphere. To a solution of **31** (2.30 g, 8.63 mmol, 1.10 eq.) in anhydrous THF (20 mL) KHMDS (0.7 M in toluene, 13.8 mL, 9.50 mmol, 1.20 eq.) was added dropwise at $-55\text{ }^\circ\text{C}$. After 1 h 10 min reaction time, aldehyde **30** (2.44 g, 7.85 mmol, 1.00 eq.) in anhydrous THF (12 mL) was added. The reaction mixture was stirred for 1 h at $-55\text{ }^\circ\text{C}$ and was then diluted with saturated aqueous NH_4Cl (80 mL). The mixture was extracted with ethyl acetate (2 x 150 mL) and the combined organic layers were washed with saturated aqueous NaCl (100 mL), dried over MgSO_4 , filtered, and concentrated under reduced pressure. The crude product was purified by flash



column chromatography (*n*-heptane/ethyl acetate 10:1 to 9:1) to give **32** (2.07 g, 5.90 mmol, 75%, *E/Z* = 94:6) as colorless oil.

Prior to the described synthesis of the Julia-Kociński-olefination, a base screening was performed on 0.650 mmol scale to evaluate isolated yield and *E/Z*-ratios (see Table S1). KHMDS was chosen due to the observed high *E*-selectivity.

Table S1. Screening of reaction conditions.

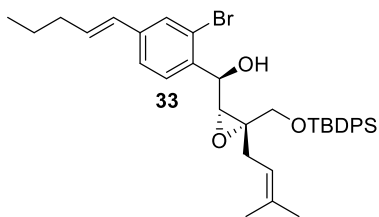
	Reaction conditions	Isolated yield	<i>E/Z</i> -ratio ^a
A	i. 31 , LDA, THF, -55 °C, 1 h 10 min ii. 30 , -55 °C, 1 h	58%	79/21
B	i. 31 , LiHMDS, THF, -55 °C, 1 h 10 min ii. 30 , -55 °C, 1 h	70%	78/22
C	i. 31 , NaHMDS, THF, -55 °C, 1 h 10 min ii. 30 , -55 °C, 1 h	72%	90/10
D	i. 31 , KHMDS, THF, -55 °C, 1 h 10 min ii. 30 , -55 °C, 1 h	60%	96/4

^a Assignment of the *E/Z*-ratios was done by integrating the ¹H-NMR signals of the alkene protons.

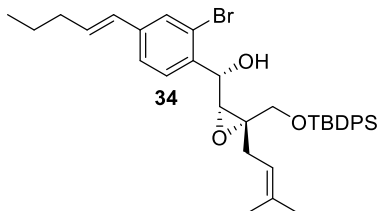
¹H-NMR (CDCl₃, 400 MHz): δ = *E*-isomer: 7.73 (d, 1H, *J* = 8.2 Hz, IC_q-CH), 7.59 (d, 1H, *J* = 2.0 Hz, BrC_q-CH), 6.95 (dd, 1H, *J* = 8.3, 2.0 Hz, IC_q-CH-CH), 6.31–6.19 (m, 2H, CH=CH), 2.21–2.14 (m, 2H, CH₂-CH=CH), 1.49 (m, 2H, CH₃-CH₂), 0.95 (t, 3H, *J* = 7.4 Hz, CH₃-CH₂); *Z*-isomer: 7.78 (d, 1H, *J* = 8.2 Hz, IC_q-CH), 7.52 (d, 1H, *J* = 2.0 Hz, BrC_q-CH), 6.89 (dd, 1H, *J* = 8.2, 2.0 Hz, IC_q-CH-CH), 6.31–6.19 (m, 1H, CH₂-CH=CH), 5.73 (td, 1H, *J* = 11.7, 7.3 Hz, CH₂-CH=CH), 2.30–2.21 (m, 2H, CH₂-CH=CH), 1.49 (m, 2H, CH₃-CH₂), 0.93 (t, 3H, *J* = 7.4 Hz, CH₃-CH₂); **¹³C-NMR** (CDCl₃, 100 MHz): δ = *E*-isomer: 140.2 (IC_q-CH), 139.9 (BrC_q), 133.7 (CH₂-CH=CH), 130.1 (BrC_q-CH), 130.0 (pentenyl-C_q), 127.9 (CH₂-CH=CH), 126.1 (IC_q-CH-CH), 98.1 (IC_q), 35.2 (CH₂-CH=CH), 22.4 (CH₃-CH₂), 13.9 (CH₃-CH₂); *Z*-isomer: 139.9 (IC_q-CH), 139.6 (BrC_q), 135.3 (CH₂-CH=CH), 132.9 (BrC_q-CH), 129.6 (pentenyl-C_q), 128.9 (IC_q-CH-CH), 126.8 (CH₂-CH=CH), 98.3 (IC_q), 30.7 (CH₂-CH=CH), 23.1 (CH₃-CH₂), 13.9 (CH₃-CH₂); **HRMS (ESI)** *m/z* calcd. for C₁₁H₁₃BrI: 350.9240 (M+H)⁺; found: no isonisation was observed; **R_f** (100% *n*-heptane): 0.72.

(*R*)-(2-Bromo-4-((*E*)-pent-1-en-1-yl)phenyl)((2*R*,3*S*)-3-(((*tert*-butyldiphenylsilyl)oxy)methyl)-3-(3-methylbut-2-en-1-yl)oxiran-2-yl)methanol (33**) and (*S*)-(2-Bromo-4-((*E*)-pent-1-en-1-yl)phenyl)((2*R*,3*S*)-3-(((*tert*-butyldiphenylsilyl)oxy)methyl)-3-(3-methylbut-2-en-1-yl)oxiran-2-yl)methanol (**34**):**

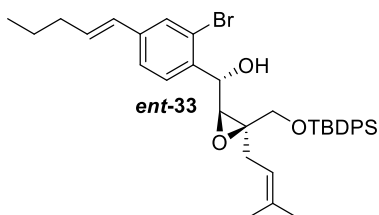
The reaction was carried out in moisture-free glassware under argon atmosphere. To a solution of iodobenzene **32** (1.73 g, 4.91 mmol, 1.20 eq.) dissolved in anhydrous THF (20 mL), *i*-PrMgCl (2.0 M in THF, 2.46 mL, 4.91 mmol, 1.20 eq.) was added dropwise at -40 °C. After stirring for 45 min, epoxy aldehyde **9** (1.67 g, 4.09 mmol, 1.00 eq.) in anhydrous THF (15 mL) was added. The reaction mixture was stirred for 1 h 30 min at -40 °C and was then diluted with saturated aqueous NH₄Cl (50 mL). The mixture was extracted with ethyl acetate (2 x 150 mL) and the combined organic layers were washed with saturated aqueous NaCl (75 mL), dried over MgSO₄, filtered, and concentrated under reduced pressure. The crude product was purified by flash column chromatography (0–15% ethyl acetate in *n*-heptane) to give addition products **33** (1.09 g, 1.72 mmol, 42% over two steps, *E/Z* ≈ 90:10) and **34** (1.09 g, 1.71 mmol, 42% over two steps, *E/Z* ≈ 95:5) as colorless oils.



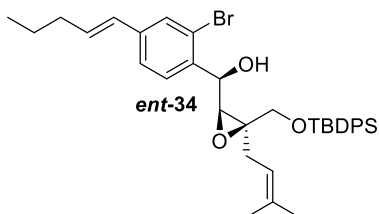
For **33**: **¹H-NMR** (CDCl₃, 400 MHz): δ = 7.75–7.68 (m, 4H, phenyl-*H*), 7.51 (d, 1H, *J* = 1.6 Hz, BrC_q-CH), 7.50–7.37 (m, 7H, pentenyl-C_q-CH-*CH* and phenyl-*H*), 7.29 (dd, 1H, *J* = 8.1, 1.6 Hz, pentenyl-C_q-CH-*CH*), 6.36–6.17 (m, 2H, CH₂=CH₂), 5.08–4.99 (m, 2H, Me₂C=CH and CH-OH), 4.01 (d, 1H, *J* = 11.1 Hz, TBDPSO-CH₂), 3.84 (d, 1H, *J* = 11.1 Hz, TBDPSO-CH₂), 3.13 (d, 1H, *J* = 7.8 Hz, epoxy-CH), 2.96 (d, 1H, *J* = 2.5 Hz, OH), 2.45 (d, 2H, *J* = 7.2 Hz, Me₂C=CH-CH₂), 2.19 (m, 2H, CH₂-CH=CH), 1.66 (s, 3H, *E*-CH₃), 1.57 (s, 3H, *Z*-CH₃), 1.50 (m, 2H, CH₃-CH₂), 1.12 (s, 9H, ^tBu-CH₃), 0.95 (t, 3H, *J* = 7.3 Hz, CH₃-CH₂); **¹³C-NMR** (CDCl₃, 100 MHz): δ = 139.6 (pentenyl-C_q), 138.2 (BrC_q-C_q), 135.9/135.8 (phenyl-CH), 135.5 (C_qMe₂), 133.0 (CH₂-CH=CH), 132.7/132.5 (phenyl-C_q), 130.2/130.2 (phenyl-CH), 130.1 (BrC_q-CH), 128.3 (CH₂-CH=CH), 128.1 (pentenyl-C_q-CH-*CH*), 128.1/128.0 (phenyl-CH), 125.5 (pentenyl-C_q-CH-*CH*), 123.1 (BrC_q), 117.8 (CH=CMe₂), 70.7 (CH-OH), 65.4 (TBDPSO-CH₂), 64.6 (epoxy-C_q), 63.9 (epoxy-CH), 35.2 (CH₂-CH=CH), 32.1 (Me₂C=CH-CH₂), 27.1 (^tBu-CH₃), 25.9 (*E*-CH₃), 22.5 (CH₃-CH₂), 19.4 (^tBu-C_q), 18.1 (*Z*-CH₃), 13.9 (CH₃-CH₂); **HRMS (ESI)** *m/z* calcd. for C₃₆H₄₄BrO₂Si: 615.2288 (M-H₂O+H)⁺; found: 615.2289 (M-H₂O+H)⁺; **R_f** (*n*-heptane/ethyl acetate 4:1): 0.44; **Specific rotation** [α]_D^{21.1} = +22.9 (*c* = 0.96; CHCl₃).



For **34**: **¹H-NMR** (CDCl₃, 400 MHz): δ = 7.72–7.61 (m, 4H, phenyl-*H*), 7.48 (d, 1H, *J* = 1.6 Hz, BrC_q-CH), 7.47–7.35 (m, 7H, pentenyl-C_q-CH-*CH* and phenyl-*H*), 7.28 (dd, 1H, *J* = 8.1, 1.6 Hz, pentenyl-C_q-CH-*CH*), 6.35–6.18 (m, 2H, CH₂=CH₂), 5.02 (m, 1H, Me₂C=CH), 4.83 (dd, 1H, *J* = 6.2, 3.7 Hz, CH-OH), 3.90 (d, 1H, *J* = 11.4 Hz, TBDPSO-CH₂), 3.80 (d, 1H, *J* = 11.5 Hz, TBDPSO-CH₂), 3.11 (d, 1H, *J* = 6.1 Hz, epoxy-CH), 2.72 (dd, 1H, *J* = 14.9, 7.9 Hz, Me₂C=CH-CH₂), 2.55 (m, 1H, OH), 2.28 (dd, 1H, *J* = 14.7, 6.9 Hz, Me₂C=CH-CH₂), 2.19 (m, 2H, CH₂-CH=CH), 1.67 (s, 3H, *E*-CH₃), 1.58 (s, 3H, *Z*-CH₃), 1.49 (m, 2H, CH₃-CH₂), 1.07 (s, 9H, ^tBu-CH₃), 0.95 (t, 3H, *J* = 7.4 Hz, CH₃-CH₂); **¹³C-NMR** (CDCl₃, 100 MHz): δ = 139.7 (pentenyl-C_q), 138.5 (BrC_q-C_q), 136.0/135.8 (phenyl-CH), 135.4 (C_qMe₂), 133.4 (CH₂-CH=CH), 133.2/133.1 (phenyl-C_q), 130.1 (BrC_q-CH), 130.0/129.9 (phenyl-CH), 128.2 (CH₂-CH=CH), 128.1 (pentenyl-C_q-CH-*CH*), 127.9/127.8 (phenyl-CH), 125.4 (pentenyl-C_q-CH-*CH*), 122.4 (BrC_q), 117.9 (CH=CMe₂), 69.4 (CH-OH), 65.5 (epoxy-C_q), 64.7 (epoxy-CH), 64.3 (TBDPSO-CH₂), 35.2 (CH₂-CH=CH), 31.4 (Me₂C=CH-CH₂), 27.0 (^tBu-CH₃), 25.9 (*E*-CH₃), 22.5 (CH₃-CH₂), 19.4 (^tBu-C_q), 18.1 (*Z*-CH₃), 13.8 (CH₃-CH₂); **HRMS (ESI)** *m/z* calcd. for C₃₆H₄₄BrO₂Si: 615.2288 (M-H₂O+H)⁺; found: 615.2298 (M-H₂O+H)⁺; **R_f** (*n*-heptane/ethyl acetate 4:1): 0.44; **Specific rotation** [α]_D^{21.1} = -6.9 (*c* = 1.01; CHCl₃).



ent-33 (0.833 g, 1.31 mmol, 37% over two steps, *E/Z* ≈ 90:10) and **ent-34** (0.947 g, 1.49 mmol, 43% over two steps, *E/Z* ≈ 95:5) were synthesized in analogous manner starting from epoxy aldehyde **ent-9** (1.43 g, 3.51 mmol) and iodide **32** (1.79 g, 5.10 mmol). The NMR data are identical to the ones reported for **33** and **34**.



For **ent-33**: **HRMS (ESI)** *m/z* calcd. for C₃₆H₄₄BrO₂Si: 615.2288 (M-H₂O+H)⁺; found: 615.2290 (M-H₂O+H)⁺; **R_f** (*n*-heptane/ethyl acetate 4:1): 0.44; **Specific rotation** [α]_D^{21.3} = -17.4 (*c* = 1.44; CHCl₃).

For **ent-34**: **HRMS (ESI)** *m/z* calcd. for C₃₆H₄₄BrO₂Si: 615.2288 (M-H₂O+H)⁺; found: 615.2298 (M-H₂O+H)⁺; **R_f** (*n*-heptane/ethyl acetate 4:1): 0.31; **Specific rotation** [α]_D^{21.3} = +2.8 (*c* = 1.71; CHCl₃).

Assignment of the stereochemistry of compound **33** and **34** was achieved *via* conclusion by analogy (Table S2). Therefore, both retardation factors of **33** and **34** (R³) and the ¹H-NMR-signals of the protons of the oxirane were compared with **12** and **13** (R²) and the intermediates **SI2** and **SI3** (R¹) of the synthesis of avicennone C.¹ The absolute stereochemistry of

¹ Kleiner, Y.; Hammann, P.; Becker, J.; Bauer, A.; Pöverlein, C.; Schuler, S. M. M. *J. Org. Chem.* **2020**, *85*, 13108–13120.

12, **13**, **SI2** and **SI3** was unambiguously determined by single X-ray crystal diffraction of crystal intermediates in the course of their total synthesis.

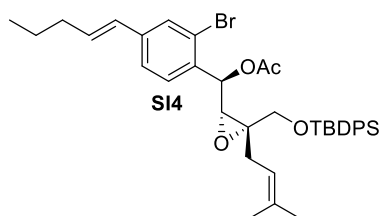
Table S2. Comparison of retardation factors and ¹H-NMR-signals of protons of the epoxide for the assignment of the stereochemistry of **33** and **34**.

	 SI2, 12 or 33			 SI3, 13 or 34		
compound	SI2 (R = R ¹)	12 (R = R ²)	33 (R = R ³)	SI3 (R = R ¹)	13 (R = R ²)	34 (R = R ³)
NMR-data ^a	3.07, d, 7.9	3.15, d, 7.8	3.13, d, 7.8	3.09, d, 6.0	3.11, d, 6.1	3.11, d, 6.1
R _f ^b	0.32	0.37	0.44	0.25	0.34	0.31

^a The NMR-spectra were recorded in CDCl₃: δ_H (400 MHz), multiplicity, *J* (epoxy-CH) in [Hz].

^b Eluent for determination of retardation factors (R_f): *n*-heptane/ethyl acetate 4:1.

(R)-(2-Bromo-4-((E)-pent-1-en-1-yl)phenyl)((2R,3S)-3-(((tert-butyl)diphenylsilyloxy)methyl)-3-(3-methylbut-2-en-1-yl)oxiran-2-yl)methyl acetate (SI4):

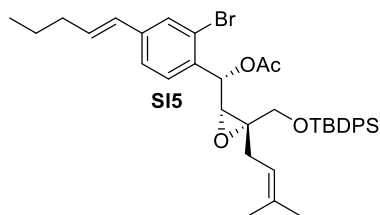


To a solution of alcohol **33** (1.07 g, 1.68 mmol, 1.00 eq.) in anhydrous DCM (20 mL) anhydrous pyridine (0.407 mL, 5.04 mmol, 3.00 eq.), Ac₂O (0.477 mL, 5.04 mmol, 3.00 eq.), and DMAP (0.021 g, 0.17 mmol, 0.10 eq.) were added. The reaction mixture was stirred for 2 h until the TLC showed complete conversion. Toluene (40 mL) was added and the reaction mixture was concentrated *in vacuo*. The crude product was purified by flash column chromatography (0–10% ethyl acetate in *n*-heptane) to give **SI4** (1.10 g, 1.63 mmol, 97%, *E/Z* > 90:10) as a colorless oil.

colorless oil.

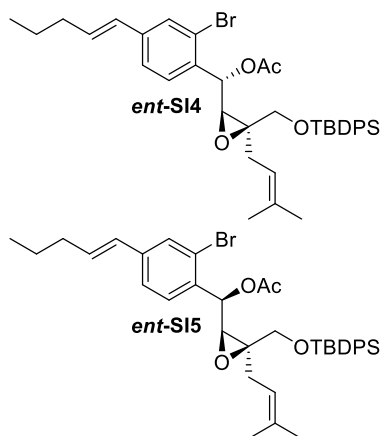
¹H-NMR (CDCl₃, 400 MHz): δ = 7.73–7.63 (m, 4H, phenyl-*H*), 7.48 (d, 1H, *J* = 1.6 Hz, BrC_q-CH), 7.46–7.29 (m, 7H, pentenyl-C_q-CH-CH and phenyl-*H*), 7.24 (dd, 1H, *J* = 8.1, 1.6 Hz, pentenyl-C_q-CH-CH), 6.33–6.16 (m, 2H, CH₂=CH₂), 5.92 (d, 1H, *J* = 7.6 Hz, CH-OAc), 5.07 (m, 1H, Me₂C=CH), 4.00 (d, 1H, *J* = 11.2 Hz, TBDPSO-CH₂), 3.86 (d, 1H, *J* = 11.2 Hz, TBDPSO-CH₂), 3.19 (d, 1H, *J* = 7.6 Hz, epoxy-CH), 2.71 (dd, 1H, *J* = 15.0, 7.7 Hz, Me₂C=CH-CH₂), 2.36 (dd, 1H, *J* = 15.0, 6.8 Hz, Me₂C=CH-CH₂), 2.18 (m, 2H, CH₂-CH=CH), 1.97 (s, 3H, CO-CH₃), 1.69 (s, 3H, *E*-CH₃), 1.61 (s, 3H, *Z*-CH₃), 1.49 (m, 2H, CH₃-CH₂), 1.07 (s, 9H, ^tBu-CH₃), 0.95 (t, 3H, *J* = 7.4 Hz, CH₃-CH₂); ¹³C-NMR (CDCl₃, 100 MHz): δ = 169.1 (C=O), 140.1 (pentenyl-C_q), 135.9/135.8 (phenyl-CH), 135.3 (BrC_q-C_q or C_qMe₂), 135.2 (BrC_q-C_q or C_qMe₂), 133.4 (CH₂-CH=CH), 133.4/133.2 (phenyl-C_q), 130.4 (BrC_q-CH), 129.8 (phenyl-CH), 128.8 (pentenyl-C_q-CH-CH), 128.2 (CH₂-CH=CH), 127.9/127.8 (phenyl-CH), 125.3 (pentenyl-C_q-CH-CH), 123.8 (BrC_q), 118.1 (CH=CMe₂), 71.2 (CH-OAc), 65.3 (epoxy-C_q), 64.1 (TBDPSO-CH₂), 62.4 (epoxy-CH), 35.2 (CH₂-CH=CH), 31.3 (Me₂C=CH-CH₂), 27.0 (^tBu-CH₃), 25.9 (*E*-CH₃), 22.5 (CH₃-CH₂), 20.9 (CO-CH₃), 19.4 (^tBu-C_q), 18.2 (*Z*-CH₃), 13.8 (CH₃-CH₂); HRMS (ESI) *m/z* calcd. for C₃₈H₄₈BrO₄Si: 675.2500 (M+H)⁺; found: 675.2515 (M+H)⁺; R_f (*n*-heptane/ethyl acetate 4:1): 0.56; Specific rotation [α]_D^{21.2} = +9.4 (c = 1.18; CHCl₃).

(S)-(2-Bromo-4-((E)-pent-1-en-1-yl)phenyl)((2R,3S)-3-(((tert-butyl)dimethylsilyloxy)methyl)-3-(3-methylbut-2-en-1-yl)oxiran-2-yl)methyl acetate (SI5):



SI5 (1.12 g, 1.65 mmol, 99%, *E/Z* ≈ 93:7) was synthesized in analogous manner starting from **34** (1.06 g, 1.67 mmol) and was obtained as colorless oil.

¹H-NMR (CDCl₃, 400 MHz): δ = 7.68–7.57 (m, 4H, phenyl-*H*), 7.45 (d, 1H, *J* = 1.6 Hz, BrC_q-CH), 7.44–7.32 (m, 6H, phenyl-*H*), 7.26 (d, 1H, *J* = 8.1 Hz, pentenyl-C_q-CH-CH), 7.21 (dd, 1H, *J* = 8.1, 1.6 Hz, pentenyl-C_q-CH-CH), 6.31–6.16 (m, 2H, CH₂=CH₂), 5.83 (d, 1H, *J* = 8.1 Hz, CH-OAc), 4.93 (m, 1H, Me₂C=CH), 3.80 (d, 1H, *J* = 11.5 Hz, TBDPSO-CH₂), 3.76 (d, 1H, *J* = 11.5 Hz, TBDPSO-CH₂), 3.34 (d, 1H, *J* = 8.1 Hz, epoxy-CH), 2.69 (dd, 1H, *J* = 15.0, 7.9 Hz, Me₂C=CH-CH₂), 2.26 (dd, 1H, *J* = 14.9, 6.7 Hz, Me₂C=CH-CH₂), 2.18 (m, 2H, CH₂-CH=CH), 2.02 (s, 3H, CO-CH₃), 1.64 (s, 3H, *E*-CH₃), 1.54 (s, 3H, *Z*-CH₃), 1.49 (m, 2H, CH₃-CH₂), 1.04 (s, 9H, ^tBu-CH₃), 0.94 (t, 3H, *J* = 7.4 Hz, CH₃-CH₂); **¹³C-NMR** (CDCl₃, 100 MHz): δ = 169.5 (C=O), 140.2 (pentenyl-C_q), 135.9/135.8 (phenyl-CH), 135.4 (C_qMe₂), 134.9 (BrC_q-C_q), 133.7 (CH₂-CH=CH), 133.5/133.1 (phenyl-C_q), 130.6 (BrC_q-CH), 129.9/129.8 (phenyl-CH), 129.1 (pentenyl-C_q-CH-CH), 128.1 (CH₂-CH=CH), 127.8/127.8 (phenyl-CH), 125.2 (pentenyl-C_q-CH-CH), 123.2 (BrC_q), 117.9 (CH=CMe₂), 73.5 (CH-OAc), 65.0 (TBDPSO-CH₂), 64.7 (epoxy-C_q), 62.4 (epoxy-CH), 35.2 (CH₂-CH=CH), 30.9 (Me₂C=CH-CH₂), 27.0 (^tBu-CH₃), 25.9 (*E*-CH₃), 22.5 (CH₃-CH₂), 21.1 (CO-CH₃), 19.4 (^tBu-C_q), 18.1 (*Z*-CH₃), 13.8 (CH₃-CH₂); **HRMS (ESI)** *m/z* calcd. for C₃₈H₄₈BrO₄Si: 675.2500 (M+H)⁺; found: 675.2515 (M+H)⁺; **R_f** (*n*-heptane/ethyl acetate 4:1): 0.42; **Specific rotation** [α]_D^{21.2} = +1.9 (*c* = 1.05; CHCl₃).

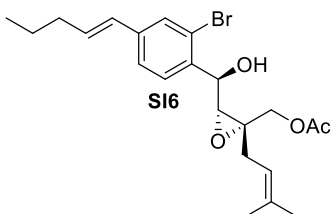


ent-SI4 (0.864 g, 1.28 mmol, 97%, *E/Z* ≈ 95:5) and **ent-SI5** (0.918 g, 1.36 mmol, 91%, *E/Z* ≈ 95:5) were synthesized in analogous manner starting from **ent-33** (0.832 g, 1.31 mmol, 1.00 eq.) and **ent-34** (0.942 g, 1.49 mmol). The NMR data are identical to the ones reported for **SI4** and **SI5**.

For **ent-SI4**: **HRMS (ESI)** *m/z* calcd. for C₃₈H₄₈BrO₄Si: 675.2500 (M+H)⁺; found: 675.2522 (M+H)⁺; **R_f** (*n*-heptane/ethyl acetate 4:1): 0.56; **Specific rotation** [α]_D^{21.2} = -5.5 (*c* = 1.26; CHCl₃).

For **ent-SI5**: **HRMS (ESI)** *m/z* calcd. for C₃₈H₄₈BrO₄Si: 675.2500 (M+H)⁺; found: 675.2518 (M+H)⁺; **R_f** (*n*-heptane/ethyl acetate 4:1): 0.42; **Specific rotation** [α]_D^{21.2} = -0.76 (*c* = 1.32; CHCl₃).

((2S,3R)-3-((R)-(2-Bromo-4-((E)-pent-1-en-1-yl)phenyl)(hydroxy)methyl)-2-(3-methylbut-2-en-1-yl)oxiran-2-yl)methyl acetate (SI6):



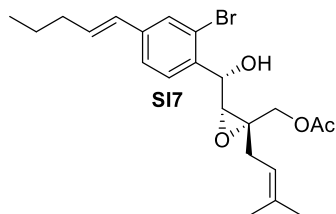
In a 50 mL falcon tube, glacial acetic acid (0.145 mL, 2.53 mmol, 1.60 eq.) followed by TBAF (1 M in THF, 2.22 mL, 2.22 mmol, 1.40 eq.) were added to a solution of TBDPS-protected alcohol **SI4** (1.07 g, 1.58 mmol, 1.00 eq.) in THF (21 mL). After 2 h, a second portion of glacial acetic acid (0.40 eq.) and TBAF (0.20 eq.) and, after further 2 h, a third portion of glacial acetic acid (0.40 eq.) and TBAF (0.20 eq.) were added to achieve complete conversion, which was monitored by TLC. The reaction mixture was loaded on silica and was then prepurified by flash column chromatography (0–20% ethyl acetate in *n*-heptane) to give an inseparable mixture of the two regioisomers with acetate on the primary or secondary hydroxy group, respectively.

To complete the acetate migration, the product mixture was dissolved in anhydrous DCM (15 mL) and DBU (0.199 mL, 1.33 mmol, 0.84 eq.) was added. After stirring at room temperature for 1 h 30 min, further DBU (0.28 eq.) was added. 2 h

later, the LC-MS indicated an acyl migration to the primary hydroxy group of > 90%.² Saturated aqueous NH₄Cl (30 mL) was added. The mixture was extracted twice with ethyl acetate (150 mL and 50 mL) and the combined organic layers were washed with saturated aqueous NaCl (50 mL), dried over MgSO₄, filtered, and concentrated under reduced pressure to yield **SI6** (0.601 g, 1.37 mmol, 87% over two steps, *E/Z* ≈ 93:7) as colorless oil.

¹H-NMR (CDCl₃, 400 MHz): δ = 7.53 (d, 1H, *J* = 1.7 Hz, BrC_q-CH), 7.50 (d, 1H, *J* = 8.1 Hz, pentenyl-C_q-CH-CH), 7.32 (dd, 1H, *J* = 8.1, 1.7 Hz, pentenyl-C_q-CH-CH), 6.36–6.18 (m, 2H, CH₂=CH₂), 5.17 (dd, 1H, *J* = 7.4, 1.7 Hz, CH-OH), 5.09 (m, 1H, Me₂C=CH), 4.39 (d, 1H, *J* = 12.0 Hz, CH₂-OAc), 4.34 (d, 1H, *J* = 12.0 Hz, CH₂-OAc), 3.19 (d, 1H, *J* = 7.4 Hz, epoxy-CH), 3.10 (d, 1H, *J* = 2.6 Hz, OH), 2.36 (m, 2H, Me₂C=CH-CH₂), 2.19 (m, 2H, CH₂-CH=CH), 2.12 (s, 3H, CO-CH₃), 1.70 (s, 3H, *E*-CH₃), 1.60 (s, 3H, *Z*-CH₃), 1.49 (m, 2H, CH₃-CH₂), 0.95 (t, 3H, *J* = 7.4 Hz, CH₃-CH₂); **¹³C-NMR** (CDCl₃, 100 MHz): δ = 171.4 (C=O), 139.8 (pentenyl-C_q), 137.9 (BrC_q-C_q), 136.0 (C_qMe₂), 133.1 (CH₂-CH=CH), 130.2 (BrC_q-CH), 128.3 (pentenyl-C_q-CH-CH), 128.2 (CH₂-CH=CH), 125.5 (pentenyl-C_q-CH-CH), 123.0 (BrC_q), 117.3 (CH=CMe₂), 69.7 (CH-OH), 65.1 (epoxy-CH), 63.6 (CH₂-OAc), 62.0 (epoxy-C_q), 35.2 (CH₂-CH=CH), 32.2 (Me₂C=CH-CH₂), 26.0 (*E*-CH₃), 22.5 (CH₃-CH₂), 21.0 (CO-CH₃), 18.1 (*Z*-CH₃), 13.8 (CH₃-CH₂); **HRMS (ESI)** *m/z* calcd. for C₂₂H₂₉BrO₄Na: 459.1141 (M+Na)⁺; found: 459.1157 (M+Na)⁺; **R_f** (*n*-heptane/ethyl acetate 2:1): 0.44; **Specific rotation** [α]_D^{21.9} = -10.9 (*c* = 1.01; CHCl₃).

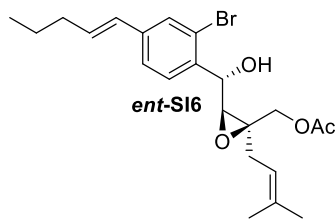
((2*S*,3*R*)-3-((*S*)-(2-Bromo-4-((*E*)-pent-1-en-1-yl)phenyl)(hydroxy)methyl)-2-(3-methylbut-2-en-1-yl)oxiran-2-yl)methyl acetate (SI7**):**



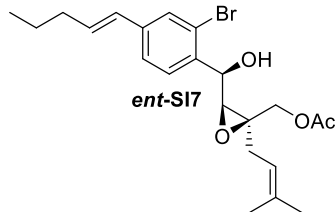
SI7 was synthesized in analogous manner starting from **SI5** (1.09 g, 1.61 mmol). Purification by flash column chromatography (0–30% ethyl acetate in *n*-heptane) after acetate migration yielded **SI7**² (0.561 g, 1.28 mmol, 79% over two steps, *E/Z* ≈ 92:8) as colorless oil.

¹H-NMR (CDCl₃, 400 MHz): δ = 7.53 (d, 1H, *J* = 1.5 Hz, BrC_q-CH), 7.49 (d, 1H, *J* = 8.1 Hz, pentenyl-C_q-CH-CH), 7.32 (dd, 1H, *J* = 8.1, 1.5 Hz, pentenyl-C_q-CH-CH), 6.37–6.20 (m, 2H, CH₂=CH₂), 5.04 (m, 1H, Me₂C=CH), 5.00 (dd, 1H, *J* = 6.5 Hz, CH-OH), 4.33 (d, 1H, *J* = 12.1 Hz, CH₂-OAc), 4.25 (d, 1H, *J* = 12.1 Hz, CH₂-OAc), 3.18 (d, 1H, *J* = 6.6, 3.2 Hz epoxy-CH), 2.69 (d, 1H, *J* = 3.4 Hz, *J* = OH), 2.55 (dd, 1H, *J* = 14.9, 7.9 Hz, Me₂C=CH-CH₂), 2.26–2.15 (m, 3H, Me₂C=CH-CH₂ and CH₂-CH=CH), 2.05 (s, 3H, CO-CH₃), 1.69 (s, 3H, *E*-CH₃), 1.57 (s, 3H, *Z*-CH₃), 1.50 (m, 2H, CH₃-CH₂), 0.95 (t, 3H, *J* = 7.4 Hz, CH₃-CH₂); **¹³C-NMR** (CDCl₃, 100 MHz): δ = 170.7 (C=O), 139.9 (pentenyl-C_q), 138.1 (BrC_q-C_q), 136.2 (C_qMe₂), 133.4 (CH₂-CH=CH), 130.2 (BrC_q-CH), 128.2 (pentenyl-C_q-CH-CH), 128.1 (CH₂-CH=CH), 125.5 (pentenyl-C_q-CH-CH), 122.5 (BrC_q), 117.0 (CH=CMe₂), 69.8 (CH-OH), 65.1 (epoxy-CH), 64.3 (CH₂-OAc), 63.1 (epoxy-C_q), 35.2 (CH₂-CH=CH), 32.0 (Me₂C=CH-CH₂), 25.9 (*E*-CH₃), 22.5 (CH₃-CH₂), 20.9 (CO-CH₃), 18.0 (*Z*-CH₃), 13.8 (CH₃-CH₂); **HRMS (ESI)** *m/z* calcd. for C₂₂H₃₀BrO₄: 437.1322 (M+H)⁺; found: 437.1335 (M+H)⁺; **R_f** (*n*-heptane/ethyl acetate 2:1): 0.41; **Specific rotation** [α]_D^{21.9} = -4.2 (*c* = 0.94; CHCl₃).

² A stagnation of the acyl migration was observed after stirring overnight and the reaction was therefore stopped. The two regioisomers could not be separated by chromatography. The minor isomer was finally removed on a later stage after TIPS-protection and saponification by flash chromatography.



ent-SI6² (0.476 g, 1.09 mmol, 87% over two steps, *E/Z* ≈ 93:7) and **ent-SI7**² (0.508 g, 1.16 mmol, 86% over two steps, *E/Z* ≈ 95:5) were synthesized in analogous manner starting from **ent-SI4** (0.844 g, 1.25 mmol) and **ent-SI5** (0.918 g, 1.36 mmol) and were obtained as colorless oils. The NMR data are identical to the ones reported for **SI6** and **SI7**.

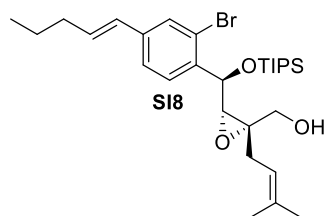


For **ent-SI6**: **HRMS (ESI)** *m/z* calcd. for C₂₂H₂₉BrO₄Na: 459.1141 (M+Na)⁺; found: 459.1140 (M+Na)⁺; **R_f** (*n*-heptane/ethyl acetate 2:1): 0.44; **Specific rotation** [α]_D^{21.2} = +14.2 (*c* = 1.34; CHCl₃).

For **ent-SI7**: **HRMS (ESI)** *m/z* calcd. for C₂₂H₂₉BrO₄Na: 459.1141 (M+Na)⁺; found: 459.1148 (M+Na)⁺; **R_f** (*n*-heptane/ethyl acetate 2:1): 0.41; **Specific rotation** [α]_D^{23.3} =

+0.8 (*c* = 1.25; CHCl₃).

((2*S*,3*R*)-3-((*R*)-(2-Bromo-4-((*E*)-pent-1-en-1-yl)phenyl)((triisopropylsilyl)oxy)methyl)-2-(3-methylbut-2-en-1-yl)oxiran-2-yl)methanol (SI8**):**



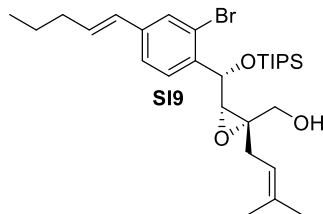
2,6-Lutidine (0.920 mL, 7.90 mmol, 4.00 eq.) and then TIPSOTf (1.06 mL, 3.95 mmol, 3.00 eq.) were added to a solution of alcohol **SI6** (0.576 g, 1.32 mmol, 1.00 eq.) in anhydrous DCM (20 mL) at 0 °C. The reaction mixture was stirred at room temperature for 2 h 30 min. Because the TLC showed remaining starting material, 2,6-lutidine (3.00 eq.) and TIPSOTf (1.50 eq.) were added. After stirring for further 1h 30 min at room temperature, the TLC showed complete conversion. Saturated aqueous NH₄Cl

(30 mL) was added. The mixture was extracted twice with ethyl acetate (150 mL and 50 mL) and the combined organic layers were washed with saturated aqueous NaCl (50 mL), dried over MgSO₄, filtered, and concentrated under reduced pressure. The TIPS and acetate protected crude product was purified twice by flash column chromatography (0–10% and 0–3% ethyl acetate in *n*-heptane).

Prepurified crude product (0.540 g, 0.91 mmol, 1.00 eq.) was dissolved in THF/H₂O (5:1, 20 mL/4 mL) and LiOH · H₂O (0.076 g, 1.82 mmol, 2.00 eq.) was added. After stirring at room temperature overnight, a second portion of LiOH · H₂O (1.11 eq.) was added to complete conversion. The reaction mixture was allowed to stir for further 1 h 30 min at room temperature until TLC showed complete conversion. Saturated aqueous NaHCO₃ (30 mL) was added. The mixture was extracted twice with ethyl acetate (100 mL and 50 mL) and the combined organic layers were washed with saturated aqueous NaCl (50 mL), dried over MgSO₄, filtered, and concentrated under reduced pressure. The crude product was purified by flash column chromatography (0–10% ethyl acetate in *n*-heptane to give **SI8** (0.335 g, 0.611 mmol, 46% over two steps, *E/Z* ≈ 95:5) as a colorless oil.

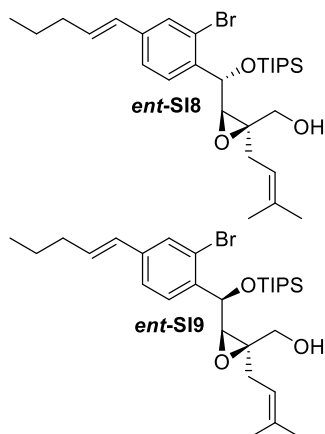
¹H-NMR (CDCl₃, 400 MHz): δ = 7.52 (d, 1H, *J* = 8.1 Hz, pentenyl-C_q-CH-CH), 7.48 (d, 1H, *J* = 1.6 Hz, BrC_q-CH), 7.32 (dd, 1H, *J* = 8.1, 1.6 Hz, pentenyl-C_q-CH-CH), 6.35–6.18 (m, 2H, CH₂=CH₂), 5.32 (d, 1H, *J* = 7.0 Hz, CH-OTIPS), 5.08 (m, 1H, Me₂C=CH), 4.01 (dd, 1H, *J* = 11.9, 5.1 Hz, CH₂-OH), 3.92 (dd, 1H, *J* = 11.9, 8.1 Hz, CH₂-OH), 3.07 (d, 1H, *J* = 7.0 Hz, epoxy-CH), 2.65 (dd, 1H, *J* = 14.6, 8.3 Hz, Me₂C=CH-CH₂), 2.19 (m, 2H, CH₂-CH=CH), 2.07 (dd, 1H, *J* = 14.6, 7.0 Hz, Me₂C=CH-CH₂), 1.82 (dd, 1H, *J* = 8.1, 5.2 Hz, OH), 1.68 (s, 3H, *E*-CH₃), 1.61 (s, 3H, *Z*-CH₃), 1.49 (m, 2H, CH₃-CH₂), 1.12–0.91 (m, 24H, TIPS-*H* and CH₃-CH₂); ¹³C-NMR (CDCl₃, 100 MHz): δ = 139.9 (BrC_q-C_q), 139.5 (pentenyl-C_q), 135.6 (C_qMe₂), 132.8 (CH₂-CH=CH), 129.9 (BrC_q-CH), 128.8 (pentenyl-C_q-CH-CH), 128.4 (CH₂-CH=CH), 125.5 (pentenyl-C_q-CH-CH), 122.4 (BrC_q), 118.1 (CH=CMe₂), 69.7 (CH-OTIPS), 67.5 (epoxy-CH), 65.5 (epoxy-C_q), 62.4 (CH₂-OH), 35.2 (CH₂-CH=CH), 32.4 (Me₂C=CH-CH₂), 25.9 (*E*-CH₃), 22.6 (CH₃-CH₂), 18.0 (*Z*-CH₃), 18.0/17.9 (CH(CH₃)₂), 13.9 (CH₃-CH₂), 12.3 (CH(CH₃)₂); **HRMS (ESI)** *m/z* calcd. for C₂₉H₄₆BrO₂Si: 533.2445 (M+H)⁺; found: 533.2456 (M+H)⁺; **R_f** (*n*-heptane/ethyl acetate 4:1): 0.41; **Specific rotation** [α]_D^{22.6} = -32.0 (*c* = 1.37; CHCl₃).

((2S,3R)-3-((S)-(2-Bromo-4-((E)-pent-1-en-1-yl)phenyl)((triisopropylsilyl)oxy)methyl)-2-(3-methylbut-2-en-1-yl)oxiran-2-yl)methanol (SI9):



SI9 (0.407 g, 0.738 mmol, 63% over two steps, *E/Z* \approx 94:6) was synthesized in analogous manner starting from **SI7** (1.06 g, 1.67 mmol) and was obtained as colorless oil.

¹H-NMR (CDCl₃, 400 MHz): δ = 7.51–7.47 (m, 2H, pentenyl-C_q-CH-CH and BrC_q-CH), 7.32 (dd, 1H, *J* = 8.1, 1.6 Hz, pentenyl-C_q-CH-CH), 6.36–6.20 (m, 2H, CH₂=CH₂), 5.12 (d, 1H, *J* = 7.8 Hz, CH-OTIPS), 4.95 (m, 1H, Me₂C=CH), 3.79 (dd, 1H, *J* = 12.1, 6.5 Hz, CH₂-OH), 3.66 (dd, 1H, *J* = 12.1, 6.5 Hz, CH₂-OH), 3.24 (d, 1H, *J* = 7.7 Hz, epoxy-CH), 2.45 (dd, 1H, *J* = 14.8, 7.7 Hz, Me₂C=CH-CH₂), 2.24–2.12 (m, 3H, CH₂-CH=CH and Me₂C=CH-CH₂), 1.69–1.60 (m, 1H, OH), 1.62 (s, 3H, *E*-CH₃), 1.55–1.44 (m, 2H, CH₃-CH₂), 1.51 (s, 3H, *Z*-CH₃), 1.17–0.92 (m, 24H, TIPS-*H* and CH₃-CH₂); **¹³C-NMR** (CDCl₃, 100 MHz): δ = 139.6 (pentenyl-C_q), 139.3 (BrC_q-C_q), 135.4 (C_qMe₂), 133.2 (CH₂-CH=CH), 130.1 (BrC_q-CH), 129.6 (pentenyl-C_q-CH-CH), 128.2 (CH₂-CH=CH), 125.5 (pentenyl-C_q-CH-CH), 121.6 (BrC_q), 117.9 (CH=CM₂), 72.3 (CH-OTIPS), 68.0 (epoxy-CH), 64.8 (epoxy-C_q), 63.3 (CH₂-OH), 35.2 (CH₂-CH=CH), 32.0 (Me₂C=CH-CH₂), 25.9 (*E*-CH₃), 22.5 (CH₃-CH₂), 18.0 (*Z*-CH₃), 18.0/17.9 (CH(CH₃)₂), 13.9 (CH₃-CH₂), 12.4 (CH(CH₃)₂); **HRMS (ESI)** *m/z* calcd. for C₂₉H₄₆BrO₂Si: 533.2445 (M+H)⁺; found: 533.2453 (M+H)⁺; **R_f** (*n*-heptane/ethyl acetate 4:1): 0.48; **Specific rotation** [α]_D^{22.6} = +11.0 (*c* = 1.18; CHCl₃).

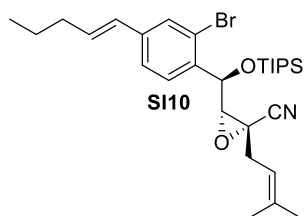


Ent-SI8 (0.267 g, 0.488 mmol, 56% over two steps, *E/Z* \approx 95:5) and **ent-SI9** (0.337 g, 0.611 mmol, 56% over two steps, *E/Z* \approx 95:5) were synthesized in analogous manner starting from **ent-SI6** (0.476 g, 1.09 mmol) and **ent-SI7** (0.480 g, 1.10 mmol). The NMR data are identical to the ones reported for **SI8** and **SI9**.

For **ent-SI8**: **HRMS (ESI)** *m/z* calcd. for C₂₉H₄₆BrO₂Si: 533.2445 (M+H)⁺; found: 533.2460 (M+H)⁺; **R_f** (*n*-heptane/ethyl acetate 4:1): 0.41; **Specific rotation** [α]_D^{21.4} = +33.0 (*c* = 1.54; CHCl₃).

For **ent-SI9**: **HRMS (ESI)** *m/z* calcd. for C₂₉H₄₆BrO₂Si: 533.2445 (M+H)⁺; found: 533.2462 (M+H)⁺; **R_f** (*n*-heptane/ethyl acetate 4:1): 0.48; **Specific rotation** [α]_D^{21.4} = -9.7 (*c* = 1.54; CHCl₃).

(2S,3R)-3-((R)-(2-Bromo-4-((E)-pent-1-en-1-yl)phenyl)((triisopropylsilyl)oxy)methyl)-2-(3-methylbut-2-en-1-yl)oxirane-2-carbonitrile (SI10):

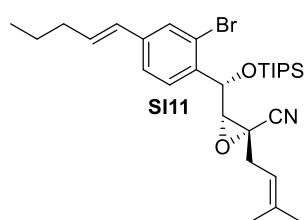


Alcohol **SI8** (0.335 g, 0.611 mmol, 1.00 eq.) was dissolved in MeCN/H₂O 9:1 (36 mL/4.0 mL) and NH₄OAc (0.187 g, 2.43 mmol, 4.00 eq.), BIAB (0.587 g, 1.82 mmol, 3.00 eq.), and TEMPO (0.019 g, 0.012 mmol, 0.20 eq.) were added. After stirring overnight at room temperature, a second portion of NH₄OAc (2.00 eq.), BIAB (1.50 eq.), and TEMPO (0.10 eq.) was added. The reaction solution was stirred further 2 h at room temperature, until the TLC and the LC-MS showed a complete conversion to the nitrile (n.b. the starting material is first converted to the intermediate aldehyde and then converted to the nitrile in a two-step fashion). Saturated aqueous Na₂SO₃ (30 mL) was added. The mixture was extracted twice with ethyl acetate (150 mL and 70 mL) and the combined organic layers were washed with saturated aqueous NaCl (50 mL), dried over MgSO₄, filtered, and concentrated under reduced pressure. The crude product was purified *via* flash column chromatography (0–3% ethyl acetate in *n*-heptane) to give nitrile **SI10** (0.158 g, 0.289 mmol, 48%, *E/Z* \approx 92:8)³ as a colorless oil.

³ The impurity was estimated to approx. 7% and could not be fully separated from the inseparable *E/Z*-mixture at this stage.

¹H-NMR (CDCl₃, 400 MHz): δ = 7.63 (d, 1H, *J* = 8.1 Hz, pentenyl-C_q-CH-CH), 7.51 (d, 1H, *J* = 1.6 Hz, BrC_q-CH), 7.34 (dd, 1H, *J* = 8.1, 1.6 Hz, pentenyl-C_q-CH-CH), 6.37–6.21 (m, 2H, CH₂=CH₂), 5.46 (d, 1H, *J* = 5.4 Hz, CH-OTIPS), 5.07 (m, 1H, Me₂C=CH), 3.16 (d, 1H, *J* = 5.4 Hz, epoxy-CH), 2.57 (dd, 1H, *J* = 14.9, 7.8 Hz, Me₂C=CH-CH₂), 2.40 (dd, 1H, *J* = 14.6, 7.0 Hz, Me₂C=CH-CH₂), 2.24–2.15 (m, 2H, CH₂-CH=CH), 1.70 (s, 3H, *E*-CH₃), 1.58 (s, 3H, *Z*-CH₃), 1.50 (m, 2H, CH₃-CH₂), 1.20–0.99 (m, 21H, TIPS-*H*), 0.96 (t, 3H, *J* = 7.4, CH₃-CH₂); **¹³C-NMR** (CDCl₃, 100 MHz): δ = 139.9 (pentenyl-C_q), 138.6 (C_qMe₂), 138.2 (BrC_q-C_q), 133.2 (CH₂-CH=CH), 130.0 (BrC_q-CH), 128.9 (pentenyl-C_q-CH-CH), 128.3 (CH₂-CH=CH), 125.5 (pentenyl-C_q-CH-CH), 122.3 (BrC_q), 117.4 (CN), 114.8 (CH=CMe₂), 70.1 (CH-OTIPS), 65.5 (epoxy-CH), 53.0 (epoxy-C_q), 35.2 (CH₂-CH=CH), 33.2 (Me₂C=CH-CH₂), 25.9 (*E*-CH₃), 22.5 (CH₃-CH₂), 18.1 (*Z*-CH₃), 18.0/17.9 (CH(CH₃)₂), 13.9 (CH₃-CH₂), 12.3 (CH(CH₃)₂); **HRMS (ESI)** *m/z* calcd. for C₂₉H₄₅BrNO₂Si: 546.2397 (M+H)⁺;)⁺; found: no ionisation was observed; **R_f** (*n*-heptane/ethyl acetate 20:1): 0.49; **Specific rotation** was not determined due to *E/Z*-mixture and significant amount of impurities.³

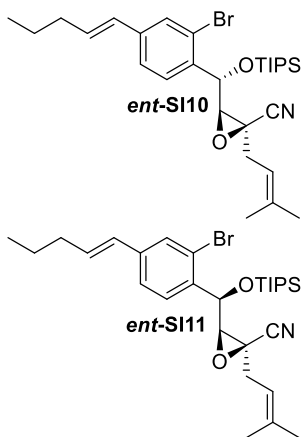
(2*S*,3*R*)-3-((*S*)-(2-Bromo-4-((*E*)-pent-1-en-1-yl)phenyl)((triisopropylsilyloxy)methyl)-2-(3-methylbut-2-en-1-yl)oxirane-2-carbonitrile (SI11):



SI11 (0.166 g, 0.304 mmol, 41%, *E/Z* ≈ 90:10)⁴ was synthesized in analogous manner starting from **SI9** (0.407 g, 0.738 mmol) and was obtained as colorless oil.

¹H-NMR (CDCl₃, 400 MHz): δ = 7.54 (d, 1H, *J* = 1.6 Hz, BrC_q-CH), 7.50 (d, 1H, *J* = 8.1 Hz, pentenyl-C_q-CH-CH), 7.32 (dd, 1H, *J* = 8.1, 1.6 Hz, pentenyl-C_q-CH-CH), 6.36–6.23 (m, 2H, CH₂=CH₂), 5.14 (d, 1H, *J* = 7.6 Hz, CH-OTIPS), 5.05 (m, 1H, Me₂C=CH), 3.38 (d, 1H, *J* = 7.7 Hz, epoxy-CH), 2.55–2.35 (m, 2H, Me₂C=CH-CH₂), 2.25–2.15 (m, 2H, CH₂-CH=CH), 1.69 (s, 3H, *E*-CH₃), 1.55 (s, 3H, *Z*-CH₃), 1.55–1.45 (m, 2H, CH₃-CH₂), 1.19–0.92 (m, 24H, TIPS-*H* and CH₃-CH₂); **¹³C-NMR** (CDCl₃, 100 MHz): δ = 140.0 (pentenyl-C_q), 138.6 (C_qMe₂), 137.6 (BrC_q-C_q), 133.4 (CH₂-CH=CH), 130.6 (BrC_q-CH), 130.0 (pentenyl-C_q-CH-CH), 128.2 (CH₂-CH=CH), 125.3 (pentenyl-C_q-CH-CH), 122.1 (BrC_q), 117.5 (CN), 114.6 (CH=CMe₂), 73.5 (CH-OTIPS), 65.9 (epoxy-CH), 53.8 (epoxy-C_q), 35.2 (CH₂-CH=CH), 33.1 (Me₂C=CH-CH₂), 25.9 (*E*-CH₃), 22.5 (CH₃-CH₂), 18.2 (*Z*-CH₃), 18.0/17.9, 13.9 (CH(CH₃)₂), 12.4 (CH(CH₃)₂); **HRMS (ESI)** *m/z* calcd. for C₂₉H₄₅BrNO₂Si: 546.2397 (M+H)⁺;)⁺; found: no ionisation was observed; **R_f** (*n*-heptane/ethyl acetate 20:1): 0.49; **Specific rotation** was not determined due to *E/Z*-mixture and significant amount of impurities.⁴

ent-SI10 (0.122 g, 0.223 mmol, 48%, *E/Z* ≈ 93:7)⁵ and **ent-SI11** (0.217 g, 0.400 mmol, 68%, *E/Z* ≈ 94:6)⁶ were synthesized in analogous manner starting from **ent-SI8** (0.255 g, 0.462 mmol) and **ent-SI9** (0.320 g, 0.580 mmol). The NMR data are identical to the ones reported for **SI10** and **SI11**.



For **ent-SI10**: **HRMS (ESI)** *m/z* calcd. for C₂₉H₄₅BrNO₂Si: 546.2397 (M+H)⁺; found: no ionisation was observed; **R_f** (*n*-heptane/ethyl acetate 20:1): 0.49; **Specific rotation** was not determined due to *E/Z*-mixture and significant amount of impurities.⁵

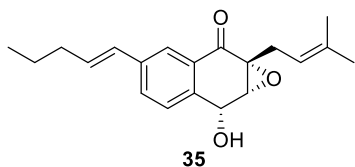
For **ent-SI11**: **HRMS (ESI)** *m/z* calcd. for C₂₉H₄₅BrNO₂Si: 546.2397 (M+H)⁺;)⁺; found: no ionisation was observed; **R_f** (*n*-heptane/ethyl acetate 20:1): 0.49; **Specific rotation** was not determined due to *E/Z*-mixture and significant amount of impurities.⁶

⁴ The impurity was estimated to approx. 17% and could not be fully separated from the inseparable *E/Z*-mixture at this stage.

⁵ The impurity was estimated to approx. 8% and could not be fully separated from the inseparable *E/Z*-mixture at this stage.

⁶ The impurity was estimated to approx. 6% and could not be fully separated from the inseparable *E/Z*-mixture at this stage.

(1a*R*,7*R*,7a*R*)-7-Hydroxy-1a-(3-methylbut-2-en-1-yl)-4-((*E*)-pent-1-en-1-yl)-7,7a-dihydronaphtho[2,3-*b*]oxiren-2(1a*H*)-one (35):

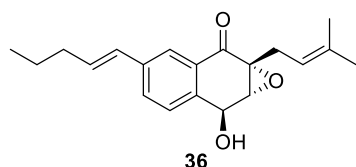


The halogen-lithium exchange was carried out in a moisture-free glassware under argon atmosphere. To a solution of nitrile **SI10** (0.080 g, 0.15 mmol, 1.00 eq.) in anhydrous THF (15 mL), prediluted *n*-BuLi solution (0.14 mmol, 1.00 eq.) was added dropwise at -100°C .⁷ The obtained orange solution was stirred for 10 min at -100°C and for 5 min without cooling bath. For hydrolysis of the intermediate imine, aqueous citric acid (10%, 80 mL) was added and the mixture was extracted with ethyl acetate (100 mL and 50 mL). The combined organic layers were washed with saturated aqueous NaHCO_3 (80 mL) as well as with saturated aqueous NaCl (80 mL), dried over MgSO_4 , filtered, and concentrated under reduced pressure. The crude product was purified by flash column chromatography (0–3% ethyl acetate in *n*-heptane), to give TIPS-protected **35** (0.059 g, 0.13 mmol) which was directly deprotected in the next step.

In a 50 mL falcon tube, glacial acetic acid (0.011 mL, 0.18 mmol, 1.40 eq.) followed by TBAF in THF (1 M in THF, 0.152 mL, 1.52 mmol, 1.20 eq.) were added to a solution of TIPS-protected **35** in THF (5 mL). After 1 h, a second portion of TBAF (0.40 eq.) and glacial acetic acid (0.60 eq.) and after further 1 h, a third portion of TBAF (0.50 eq.) and glacial acetic acid (0.70 eq.) were added to complete conversion of the starting material which was monitored by TLC. The reaction mixture was allowed to stir overnight at room temperature and was then loaded on silica and filtered through a small silica column (25% ethyl acetate in *n*-heptane). The product containing fractions were collected, concentrated *in vacuo* and the residue was purified *via* flash column chromatography (0–35% ethyl acetate in *n*-heptane) to give **35** (0.027 g, 0.085 mmol, 58% over two steps, *E/Z* \approx 89:11) as colorless oil.

¹**H-NMR** (CDCl_3 , 400 MHz): δ = 7.86–7.83 (m, 1H, pentenyl- C_q -CH- C_q), 7.59–7.55 (m, 2H, pentenyl- C_q -CH-CH and pentenyl- C_q -CH-CH), 6.43–6.25 (m, 2H, $\text{CH}_2=\text{CH}_2$), 5.10 (m, 1H, $\text{Me}_2\text{C}=\text{CH}$), 5.08–5.01 (m, 1H, CH-OH), 3.83 (d, 1H, J = 2.2 Hz, epoxy-CH), 2.92 (dd, 1H, J = 15.3, 7.9 Hz, $\text{Me}_2\text{C}=\text{CH}-\text{CH}_2$), 2.61 (dd, 1H, J = 15.4, 7.0 Hz, $\text{Me}_2\text{C}=\text{CH}-\text{CH}_2$), 2.43 (s, br, 1H, OH), 2.20 (m, 2H, $\text{CH}_2-\text{CH}=\text{CH}$), 1.73 (s, 3H, *E*- CH_3), 1.68 (s, 3H, *Z*- CH_3), 1.50 (m, 2H, CH_3-CH_2), 0.95 (t, 3H, J = 7.4 Hz, CH_3-CH_2); ¹³**C-NMR** (CDCl_3 , 100 MHz): δ = 194.2 (C=O), 138.5 (pentenyl- C_q), 138.1 (C_q -CH-OH), 136.4 (C_qMe_2), 133.1 ($\text{CH}_2-\text{CH}=\text{CH}$), 131.7 (pentenyl- C_q -CH-CH), 129.1 (Ar- C_q -CO), 128.6 ($\text{CH}_2-\text{CH}=\text{CH}$), 127.4 (pentenyl- C_q -CH-CH), 124.6 (pentenyl- C_q -CH- C_q), 116.6 ($\text{CH}=\text{CMe}_2$), 66.3 (CH-OH), 62.5 (epoxy- C_q), 59.6 (epoxy-CH), 35.2 ($\text{CH}_2-\text{CH}=\text{CH}$), 26.8 ($\text{Me}_2\text{C}=\text{CH}-\text{CH}_2$), 26.0 (*E*- CH_3), 22.5 (CH_3-CH_2), 18.2 (*Z*- CH_3), 13.8 (CH_3-CH_2); **HRMS (ESI)** *m/z* calcd. for $\text{C}_{20}\text{H}_{25}\text{O}_3$: 313.1798 (M+H)⁺; found: 313.1800 (M+H)⁺; **R_f** (*n*-heptane/ethyl acetate 2:1): 0.41; **Specific rotation** $[\alpha]_D^{24.0} = +212.9$ (c = 0.63; CHCl_3).

(1a*R*,7*S*,7a*R*)-7-Hydroxy-1a-(3-methylbut-2-en-1-yl)-4-((*E*)-pent-1-en-1-yl)-7,7a-dihydronaphtho[2,3-*b*]oxiren-2(1a*H*)-one (36):

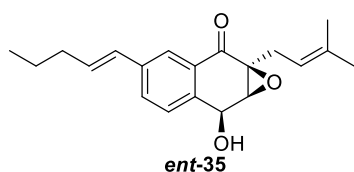


36 (0.017 g, 0.054 mmol, 31% over two steps, *E/Z* \approx 90:10) was synthesized in analogous manner starting from **SI11** (0.093 g, 0.17 mmol) and was obtained as colorless oil.

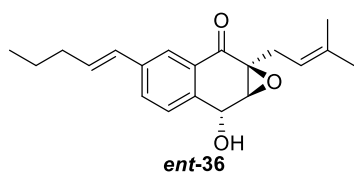
¹**H-NMR** (CDCl_3 , 400 MHz): δ = 7.74 (d, 1H, J = 1.8 Hz, pentenyl- C_q -CH- C_q), 7.51 (dd, 1H, J = 8.0, 1.9 Hz, pentenyl- C_q -CH-CH), 7.33 (d, 1H, J = 7.9 Hz, pentenyl- C_q -CH-CH), 6.41–6.21 (m, 2H, $\text{CH}_2=\text{CH}_2$), 5.17 (d, 1H, J = 2.0 Hz, CH-OH), 5.13 (m, 1H, $\text{Me}_2\text{C}=\text{CH}$), 3.82 (d, 1H, J = 2.3 Hz, epoxy-CH), 2.90 (dd, 1H, J = 15.4, 7.8 Hz, $\text{Me}_2\text{C}=\text{CH}-\text{CH}_2$), 2.66 (dd, 1H, J = 15.4, 6.7 Hz, $\text{Me}_2\text{C}=\text{CH}-\text{CH}_2$), 2.43 (s, br, 1H, OH), 2.20 (m, 2H, $\text{CH}_2-\text{CH}=\text{CH}$), 1.73 (s, 3H, *E*- CH_3), 1.69 (s, 3H, *Z*- CH_3), 1.50 (m, 2H, CH_3-CH_2), 0.96 (t, 3H, J = 7.4 Hz, CH_3-CH_2); ¹³**C-NMR** (CDCl_3 , 100 MHz): δ = 195.2 (C=O), 139.5 (pentenyl- C_q), 137.8 (C_q -CH-OH), 136.3 (C_qMe_2), 133.5 ($\text{CH}_2-\text{CH}=\text{CH}$), 131.7 (pentenyl- C_q -CH-CH), 130.0 (pentenyl- C_q -CH-CH), 129.8 (Ar- C_q -CO), 128.6 ($\text{CH}_2-\text{CH}=\text{CH}$), 124.7 (pentenyl- C_q -CH- C_q), 116.7 ($\text{CH}=\text{CMe}_2$),

⁷ A 2.5 M solution of *n*-BuLi in hexane from a commercially supplier was diluted by a factor of four at -78°C with anhydrous THF for a better handling. For the -100°C cooling bath a mixture of ethanol and liquid N_2 was used.

66.9 (CH-OH), 60.7 (epoxy-C₃), 60.2 (epoxy-CH), 35.2 (CH₂-CH=CH), 26.7 (Me₂C=CH-CH₂), 26.0 (*E*-CH₃), 22.5 (CH₃-CH₂), 18.2 (*Z*-CH₃), 13.9 (CH₃-CH₂); **HRMS (ESI)** *m/z* calcd. for C₂₀H₂₅O₃: 313.1798 (M+H)⁺; found: 313.1802 (M+H)⁺; **R_f** (*n*-heptane/ethyl acetate 2:1): 0.38; **Specific rotation** [α]_D^{24.0} = +145.3 (c = 0.36; CHCl₃).



***Ent*-35** (0.007 g, 0.02 mmol, 16% over two steps, *E/Z* ≈ 93:7) and ***ent*-36** (0.009 g, 0.03 mmol, 19% over two steps, *E/Z* ≈ 99:1) were synthesized in analogous manner starting from ***ent*-SI10** (0.074 g, 0.13 mmol) and ***ent*-SI11** (0.081 g, 0.15 mmol). The NMR data are identical to the ones reported for **35** and **36**.



For ***ent*-35**: **HRMS (ESI)** *m/z* calcd. for C₂₀H₂₅O₃: 313.1798 (M+H)⁺; found: 313.1808 (M+H)⁺; **R_f** (*n*-heptane/ethyl acetate 2:1): 0.41; **Specific rotation** [α]_D^{21.9} = -149.6 (c = 0.35; CHCl₃).

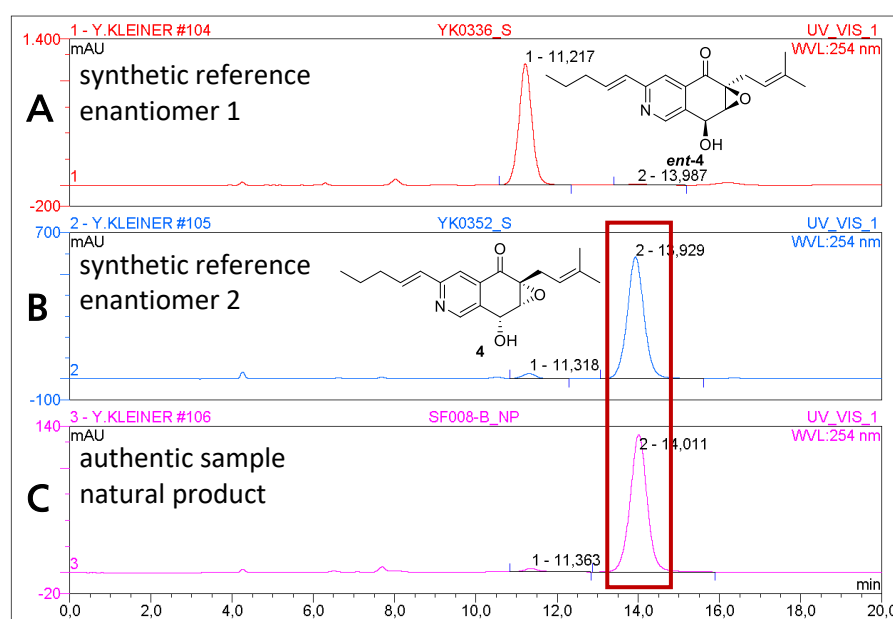
For ***ent*-36**: **HRMS (ESI)** *m/z* calcd. for C₂₀H₂₅O₃: 313.1798 (M+H)⁺; found: 313.1808 (M+H)⁺; **R_f** (*n*-heptane/ethyl acetate 2:1): 0.38; **Specific rotation** [α]_D^{21.9} = -191.0 (c = 0.60; CHCl₃).

1.2. Confirmation of the absolute stereochemistry of (+)-floyocidin B (4) by chiral HPLC

Operator:HPLC-A Timebase:HPLC_1 Sequence:Y.KLEINER

Page 1-1
1.12.2020 2:21 PM

106 SF008-B_NP		CHIRALPAK IC 1ml/min. 85% Hexan, 15% IPA	
Sample Name:	SF008-B_NP	Injection Volume:	5,0
Vial Number:	108	Channel:	UV_VIS_1
Sample Type:	unknown	Wavelength:	254
Control Program:	Analyse03_F10_A85_B00_C15	Bandwidth:	1
Quantif. Method:	default	Dilution Factor:	1,0000
Recording Time:	1.12.2020 13:28	Sample Weight:	1,0000
Run Time (min):	20,00	Sample Amount:	1,0000



No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	11,36	n.a.	3,245	1,670	2,54	n.a.	BMB
2	14,01	n.a.	131,368	63,949	97,46	n.a.	BMB
Total:			134,613	65,618	100,00	0,000	

default/Integration

Chromeleon (c) Dionex 1996-2001
Version 6.80 SR15 Build 4656 (243203)

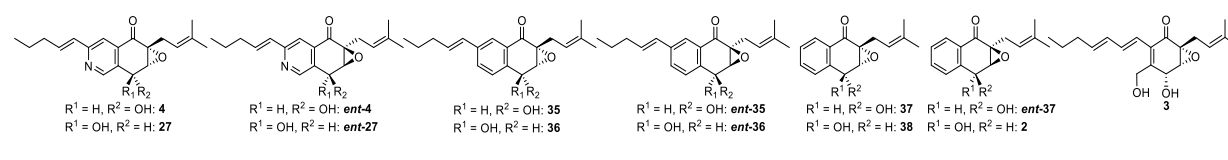
Figure S1. Chiral HPLC analysis for confirmation of correct absolute configuration *via* comparison of isolated NP (C) with synthesized enantiomers (A and B).

2. Biological and medicinal-chemical profiling

2.3. Antimicrobial activities

Growth inhibitory potency was determined by micro-broth dilution assays. Each compound was dissolved in DMSO and tested in triplicate. For assays against *E. coli* ATCC25922, *P. aeruginosa* ATCC27859 and *S. aureus* ATCC25923, pre-cultures were incubated overnight at 37 °C and 180 rpm before being diluted to a concentration of $5 \cdot 10^5$ cells/mL in cation adjusted Mueller Hinton II medium (CAMH). The cell suspension for *E. coli* ATCC25922 was additionally prepared in CAMH supplemented with 44 mM sodium bicarbonate. Dilution series of rifampicin (Sigma), tetracycline (Sigma), and gentamycin (Sigma) were used as positive controls (64-0.03 µg/mL). After incubation for 18 h (37 °C, 180 rpm, 85% relative humidity (r.H.)), cell growth was assessed by measuring the turbidity with a microplate spectrophotometer at 600 nm (LUMIstar® Omega BMG Labtech). The pre-culture of *M. smegmatis* ATCC607 was incubated in Brain-Heart Infusion broth for 48 h at 37 °C and 180 rpm. The assay cell density was adjusted as described above in CAMH. Isoniazid was used instead of gentamycin as a third positive control. *M. smegmatis* assays were incubated for 48 h (37 °C, 180 rpm, 85% r.H.) before cell viability was assessed *via* ATP quantification (BacTiter-Glo™, Promega) according to the manufacturer's instructions. Assays against *M. tuberculosis* H37Rv were carried out in the L3 approved laboratories of Evotec (Lyon, France). Tests were done in Middlebrook 7H9 broth medium and incubated at 37 °C and 5% CO₂ for 6 days. Bacterial viability was determined by CellTiter-Blue™ (Promega) according to the manufacturer's instructions. *C. albicans* FH2173 was incubated for 48 h at 28 °C and 180 rpm and diluted to $1 \cdot 10^6$ cells/mL in CAMH. Assays were incubated for 48 h at 37 °C and 180 rpm. Tebuconazole (Cayman Chemical Company), amphotericin B (Sigma), and nystatin (Sigma) were used as positive control (64–0.03 µg/mL). Readout was done by ATP quantification. Compound showing at least 80% relative growth inhibition of the indicator strain were considered bioactive at the respective concentration. Compounds exhibiting no activity at 128 µg/mL were scored "not active" (NA).

Table S3. Bioactivity data of synthesized NP and SAR-compounds against several pathogens.^a



Compound	MIC [µg/mL]												
	4	27	ent-4	ent-27	35	36	ent-35	ent-36	37	38	ent-37	2	3
<i>E. coli</i> ATCC25922	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
<i>E. coli</i> ATCC25922 MHC	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	128	NA	NA
<i>P. aeruginosa</i> ATCC27853	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
<i>S. aureus</i> ATCC25923	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	64
<i>M. catarrhalis</i> ATCC252381	16	16–8	16	8	64	8	32	16–8	128	128	64–32	64	32–16
<i>C. albicans</i> FH2173	NA	NA	NA	NA	NA	NA	NA	NA	128	NA	128	128	NA

^a NA = not active.

2.4. Assay descriptions for the profiling of all four stereoisomers of (+)-floyocidin B and (-)-avicennone C,¹ the avicennone C-floyocidin B hybrids 35, *ent*-35, 36 and *ent*-36 as well as the natural product (+)-floyocidin A.

2.4.1. Determination of logD_{7.4}

LogD values at pH 7.4 were determined by a standardized HPLC method derived from by Genieser *et al.*⁸ The calculation of the logD value for measured compounds is performed by comparison of the retention times with standard compounds of known distribution coefficients between 1-octanol and water at pH 7.4.

2.4.2. Microsomal stability

Pooled liver microsomes are purchased. Microsomes are stored at -80 °C prior to use. Microsomes (final protein concentration 0.5 mg/mL), 0.1 M phosphate buffer pH 7.4, and test compound (final substrate concentration 1 µM; final DMSO concentration 0.25%) are pre-incubated at 37 °C prior to the addition of NADPH (final concentration 1 mM) to initiate the reaction. A minus cofactor control incubation is included for each compound tested where 0.1 M phosphate buffer pH 7.4 is added instead of NADPH. Two control compounds are included. All incubations are performed singularly for each test compound. Each compound is incubated for 0, 5, 15, 30, and 45 min. The control is incubated for 45 min only. The reactions are stopped by transferring incubate into acetonitrile at the appropriate time points, in a 1:3 ratio. The termination plates are centrifuged at 3,000 rpm for 20 min at 4 °C to precipitate the protein.

From a plot of ln peak area ratio (compound peak area/internal standard peak area) against time, the gradient of the line is determined. Subsequently, half-life and intrinsic clearance are calculated using the equations below:

Elimination rate constant (k) = (- gradient)

$$\text{Half-life } (t_{1/2})(\text{min}) = \frac{0.693}{k}$$

$$\text{Intrinsic clearance } (CL_{\text{int}})(\mu\text{L}/\text{min}/\text{mg protein}) = \frac{V \times 0.693}{t_{1/2}}$$

where V = Incubation volume (µL)/Microsomal protein (mg)

Relevant control compounds are assessed, ensuring intrinsic clearance values fall within the specified limits (if available).

2.4.3. Cellular permeability assay utilizing Caco2 cell line

Caco-2 cells obtained from the ATCC are used between passage numbers 40 - 60. Cells are seeded onto Transwell plates at 1×10^5 cells/cm². The cells are cultured in DMEM and media is changed every two or three days. On day 20 the permeability study is performed. Cell culture and assay incubations are carried out at 37 °C in an atmosphere of 5% CO₂ with a relative humidity of 95%. On the day of the assay, the monolayers are prepared by rinsing both apical and basolateral surfaces twice with Hanks Balanced Salt Solution (HBSS) at the desired pH warmed to 37 °C. Cells are then incubated with HBSS at the desired pH in both apical and basolateral compartments for 40 min to stabilize physiological parameters. The dosing solutions are prepared by diluting test compound with assay buffer to give the desired test compound incubation concentration (10 µM). At 120 min the apical compartment inserts and the companion plates are separated and apical and basolateral samples diluted for analysis. Test compound permeability is assessed in duplicate. Test and control compounds are quantified by LC-MS/MS cassette analysis using a 7-point calibration with appropriate dilution of the samples. The starting concentration (C₀) is determined from the dosing solution and the experimental recovery calculated

⁸ Krass, J. D., Jastorff, B., and Genieser, H.-G. *Anal. Chem.* **1997**, *69*, 2575–2581.

from C_0 and both apical and basolateral compartment concentrations. Four control compounds are screened alongside the test compounds.

The permeability coefficient (P_{app}) for each compound is calculated from the following equation:

$$P_{app} = \left(\frac{dQ/dt}{C_0 \times A} \right)$$

Where dQ/dt is the rate of permeation of the drug across the cells, C_0 is the donor compartment concentration at time zero and A is the area of the cell monolayer. C_0 is obtained from analysis of the dosing solution.

For bi-directional experiments, an efflux ratio (ER) is calculated from mean A-B and B-A data. This is derived from:

$$ER = \frac{P_{app(B-A)}}{P_{app(A-B)}}$$

Table S4. Results for cellular permeability assays utilizing Caco2 cell lines with standard deviations (SD) for P_{app} .

P_{app} [10^{-7} cm s $^{-1}$] (recovery [%])				
	A2B		+ Elacridar	
3	(a)	(b)		
4	41.1 (10.1)	SD: 3.78	56.2 (14.2)	SD: 6.55
27	117 (42.1)	SD: 3.47	87.7 (38.5)	SD: 3.40
ent-4	73.5 (23.4)	SD: 9.38	46.7 (28.7)	SD: 3.12
ent-27	40.5 (8.69)	SD: 3.56	64.4 (15.3)	SD: 3.71
35	135 (76.0)	SD: 26.2	181 (29.6)	(c)
36	(a) (16.1)		(a) (19.9)	
ent-35	162 (37.2)	(c)	(a) (46.1)	
ent-36	8.55 (14.3)	SD: .16	12.4 (12.0)	SD: 9.83
37	326 (74.8)	SD: 22.5	279 (80.9)	SD: 5.39
38	620 (127)	SD: 17.4	331 (132)	SD: 156
ent-37	360 (81.3)	SD: 23.6	300 (74.6)	SD: 6.89
2	407 (86.7)	SD: 22.3	393 (83.4)	SD: 9.70

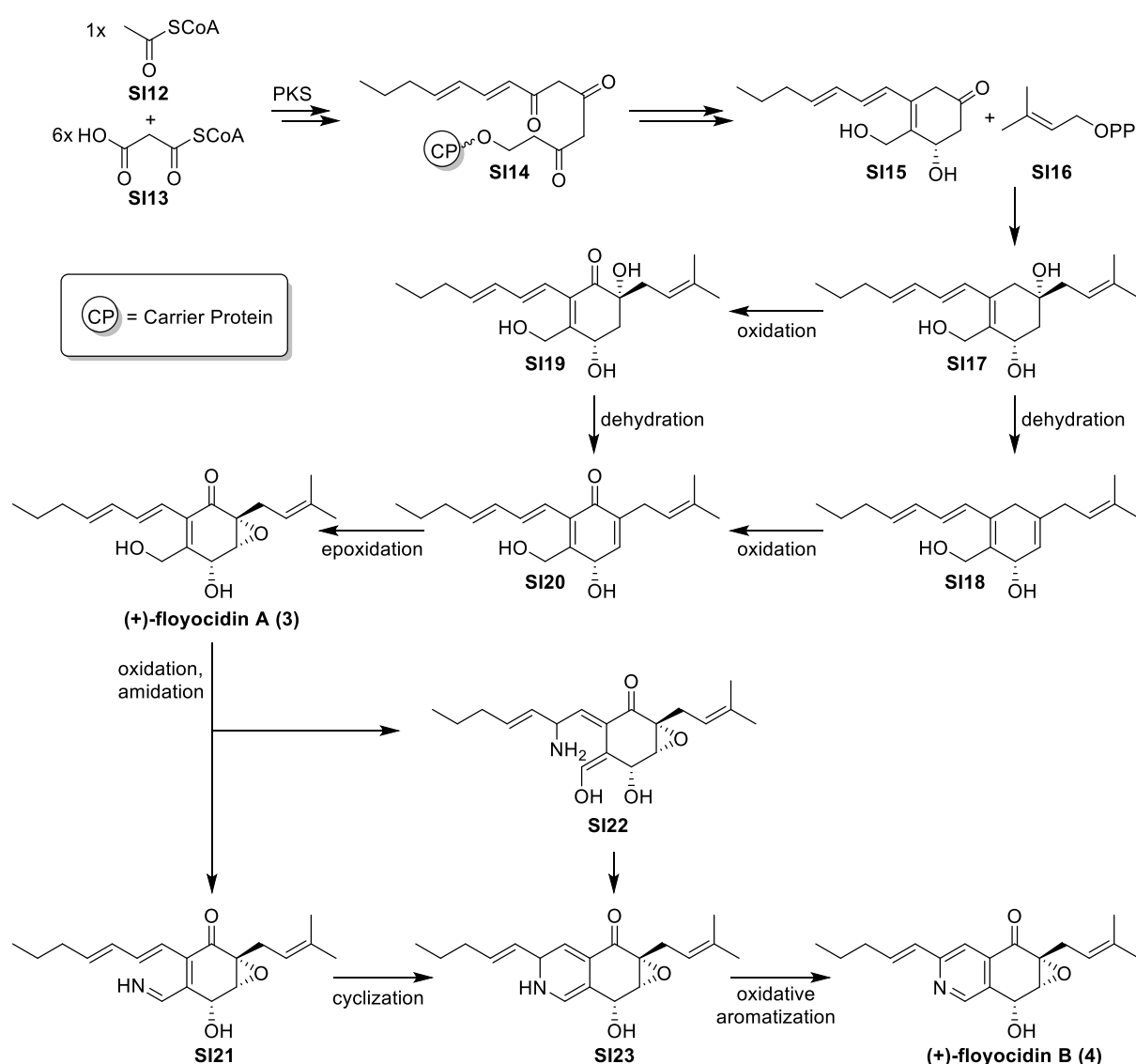
[a] No data obtained. [b] Not determined. [c] Only one value obtained.

2.4.4. Cytotoxicity on THP1 and HepG2 cells

After 4 hours of HepG2 cell line (provided by ATCC) plating at 2,500 cells /well in 384-w plates, compounds were added to the cells. After an incubation at 37 °C for 40 h, the CellTiterGlo assay (provided by Promega) was performed. Luminescence measurement is related to the cell viability. Compound activity is determined by using the control wells with no treatment as control (100% of growth). The assay was performed identically with THP1 cells. All incubations were performed in duplicate.

3. Biosynthetic hypothesis of floycidins

The structure of the floycidins indicates a polyketide synthase (PKS)-dependent biosynthesis.⁹ Hence, as proposed for (+)-ambuic acid (**1**) and its derivatives,¹⁰ the backbone structure can be build up from one acetyl-CoA (**SI12**) and six malonyl-CoA (**SI13**) building blocks (Scheme S1). The nascent linear polyketide chain **SI14** undergoes an intramolecular cyclization that can be catalyzed by a cyclase, resulting in the six-membered ring present in (+)-floycidin A (**3**). In contrast to (+)-ambuic acid (**1**), an additional *E*-double bond in the hepta-1,3-dien-1-yl chain is located, indicating ketoreduction and dehydration taking place at the second acetate unit, yielding this C=C double bond. The prenyl side chain is presumably acetyl-CoA-derived, too. It can be deduced from dimethylallyl pyrophosphate (DMAPP, **SI16**), which is an isomer of isopentenyl pyrophosphate (IPP). Furthermore, an oxygenase introduces the keto group to the ring. The epoxide of **3** is expected to result from an epoxidase-catalyzed oxidation of the corresponding double bond. The order of the decorating steps remains unclear until now. The primary hydroxyl group of **3** represents the position of attachment to the carrier protein during polyketide assembly.



Scheme S1. Plausible biosynthetic pathway of (+)-floycidin A and (+)-floycidin B.

⁹ Yuan, C.; Ding, G.; Wang, H.-Y.; Guo, Y.-H.; Shang, H.; Ma, X.-J.; Zou, Z.-M. *BioMed Res. Int.* **2017**, 6961928.

¹⁰ Yu, X.; Gao, Y.; Frank, M.; Mándi, A.; Kurtán, T.; Müller, W. E. G.; Kalscheuer, R.; Guo, Z.; Zou, K.; Liu, Z.; Proksch, P. *Tetrahedron* **2021**, 79, 131876.

For the formation of the dihydroisoquinolinone scaffold of (+)-floyocidin B (**4**), a nitrogen atom has to be introduced. Hence, the putative precursor **SI23** could be accessible by a selective oxidation of the terminal hydroxyl group and followed by an amidation-cyclization cascade. Alternatively, an amidase could add an ammonia equivalent to the hepta-1,3-dien-1-yl side chain, which then could be oxidatively cyclized. Final oxidative aromatization would complete the proposed biosynthesis of (+)-floyocidin B (**4**).

4. Crystallographic data collection and refinement details

Diffraction data were collected at low temperatures (100K) using φ - and ω -scans on a BRUKER D8 Venture System equipped with dual μ S microfocus sources, a PHOTON100 detector and an OXFORD CRYOSYSTEMS 700 low temperature system. Mo- K_{α} radiation with a wavelength of 0.71073 Å and a collimating Quazar multilayer mirror were used.

Semi-empirical absorption corrections from equivalents were applied using SADABS.¹¹ The structure was solved by direct methods using SHELXT¹² and refined against F^2 on all data by full-matrix least squares using SHELXL.¹³ All non-hydrogen atoms were refined anisotropically O-H hydrogen atoms were located in the Fourier difference map and set to ideal distances and C-H hydrogen atoms were positioned at geometrically calculated positions and refined using a riding model. The isotropic displacement parameters of all hydrogen atoms were fixed to 1.2x or 1.5x (CH_3 and OH hydrogens) the U_{eq} value of the atoms they are linked to. The crystallographic data were deposited with the Cambridge Crystallographic Database as 2075537 and can be obtained free of charge.¹⁴

The structure of **17** was solved in the orthorhombic space group $C22_1$. The asymmetric unit contains one molecule of **17**, exhibiting full molecule disorder. The disorder was modelled with the help of same distances restraints, similarity restraints on anisotropic displacement parameters, restraints to a common plane¹⁵ and advanced rigid bond restraints.¹⁶ Some disordered atoms with close coordinates were set to the same anisotropic displacement parameters. The disorder ratio was allowed to refine freely and converged to 0.520(2). The absolute structure was confirmed by classical Flack x (0.011(8)) and the Parsons parameter (0.014(2)).¹⁷

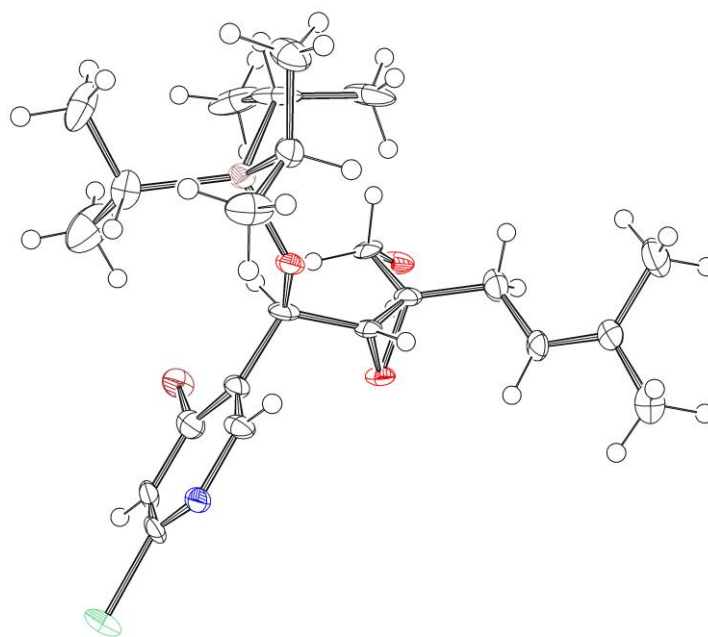


Figure S2. Thermal ellipsoid plot of the molecular structure of **17**. Thermal ellipsoid probability set to 50%, only most occupied disorder part shown.

¹¹ Krause, L.; Herbst-Irmer, R.; Sheldrick, G. M.; Stalke, D. J. *Appl. Cryst.* **2015**, *48*, 3–10.

¹² Sheldrick, G. M. *Acta Cryst. A* **2015**, *71*, 3–8.

¹³ Sheldrick, G. M. *Acta Cryst. C* **2015**, *71*, 3–8.

¹⁴ <https://www.ccdc.cam.ac.uk/structures/>

¹⁵ Müller, P. *Cryst. Rev.* **2009**, *15*, 57–83.

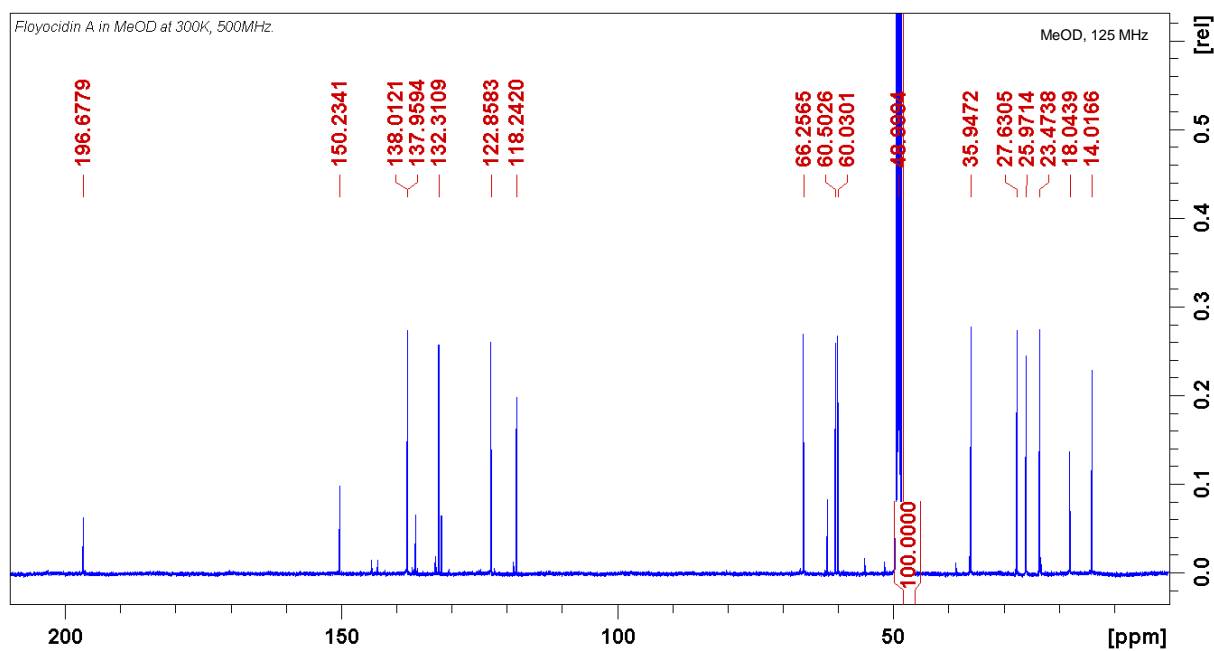
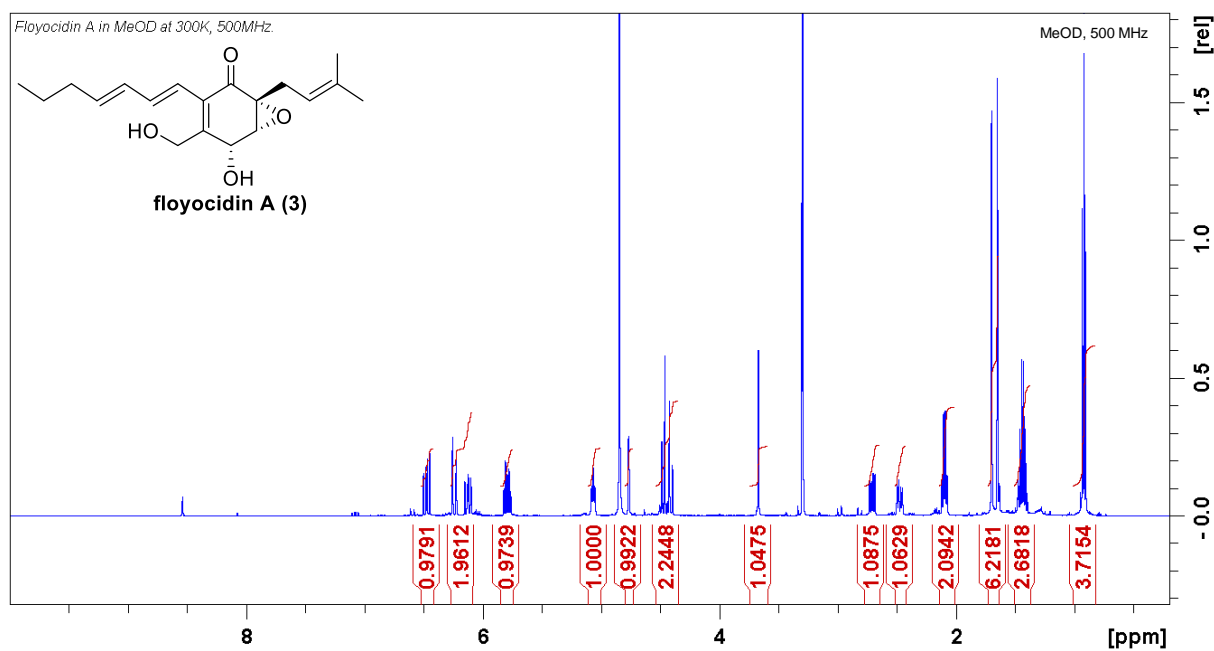
¹⁶ Thorn, A.; Dittrich, B.; Sheldrick, G. M. *Acta Cryst. A* **2012**, *68*, 448–451.

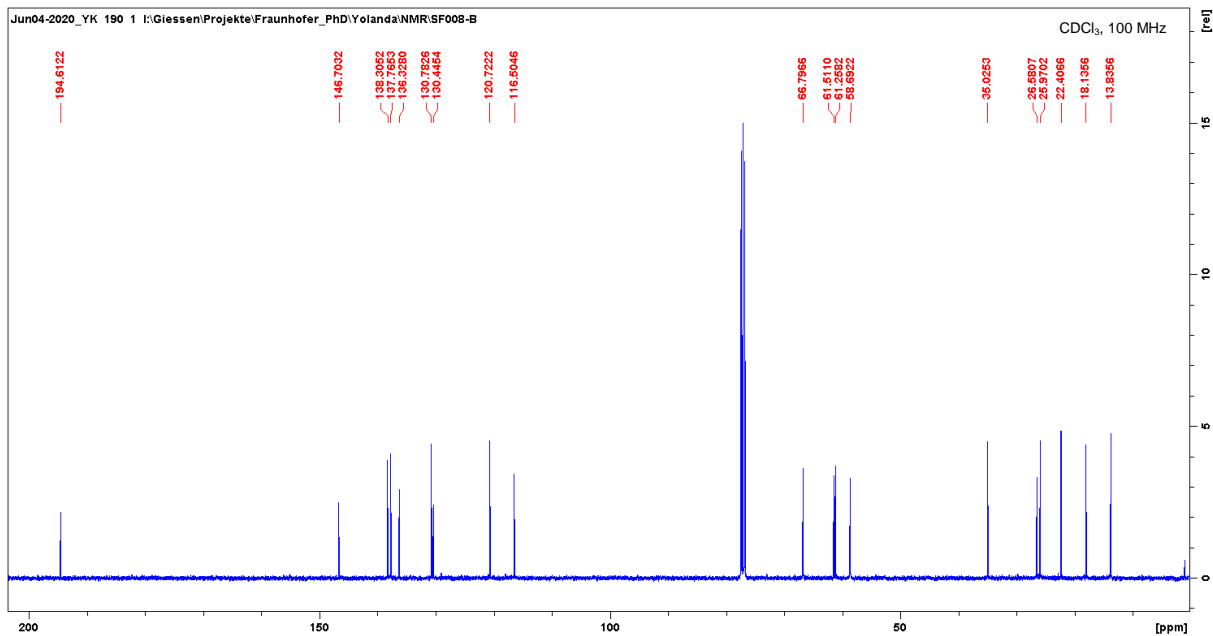
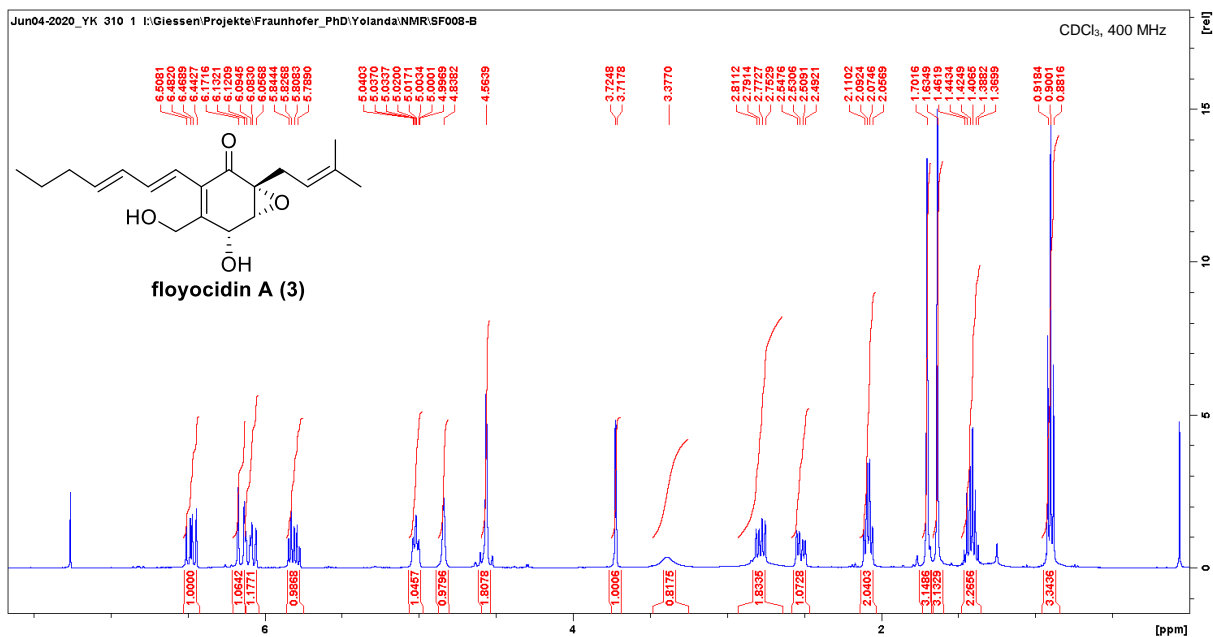
¹⁷ Parsons, H.; Flack, H. D.; Wagner, T. *Acta Cryst. A* **2013**, *69*, 249–259.

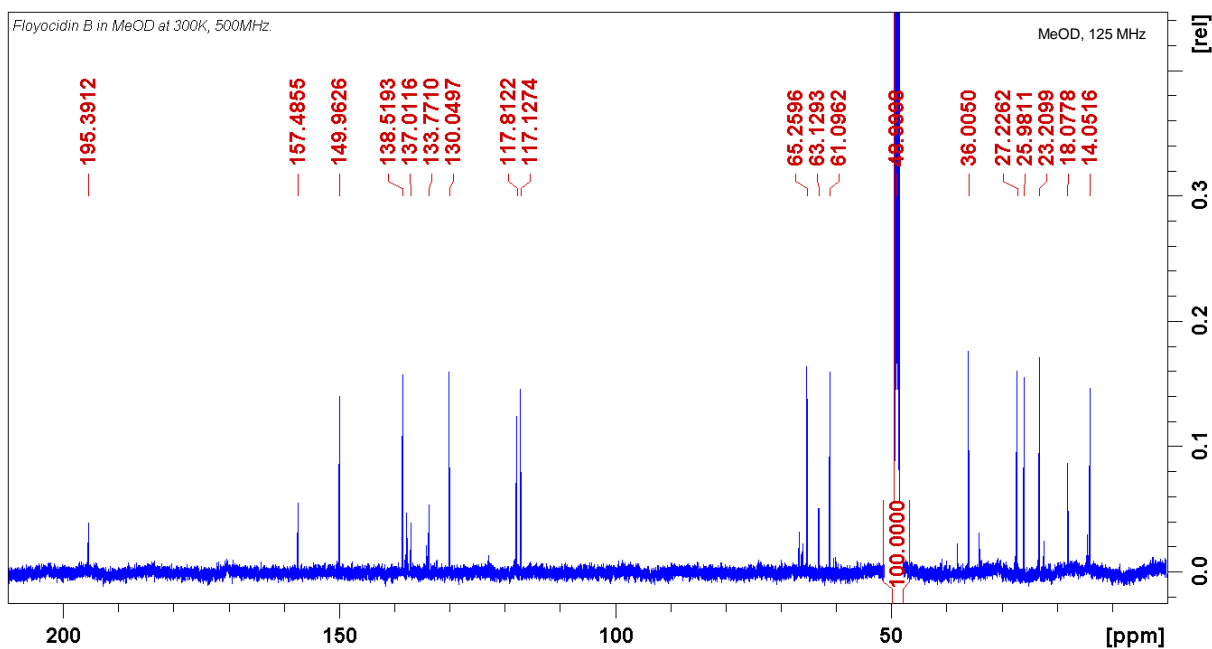
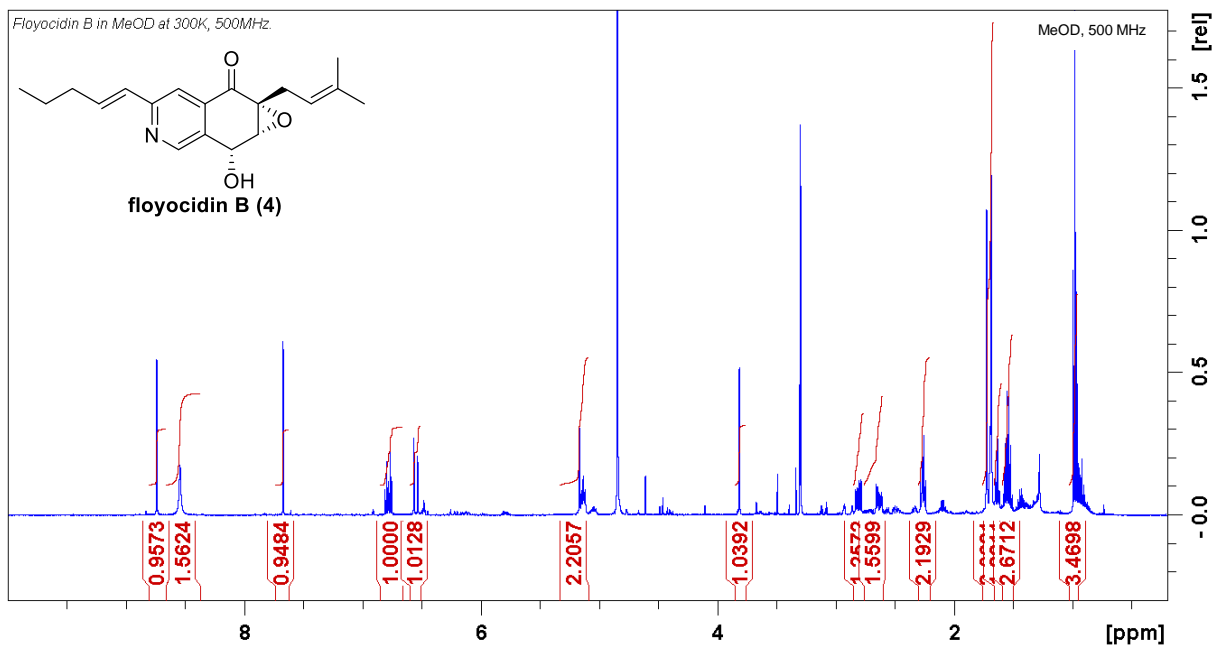
Table S5. Crystal data and structure refinement for 17.

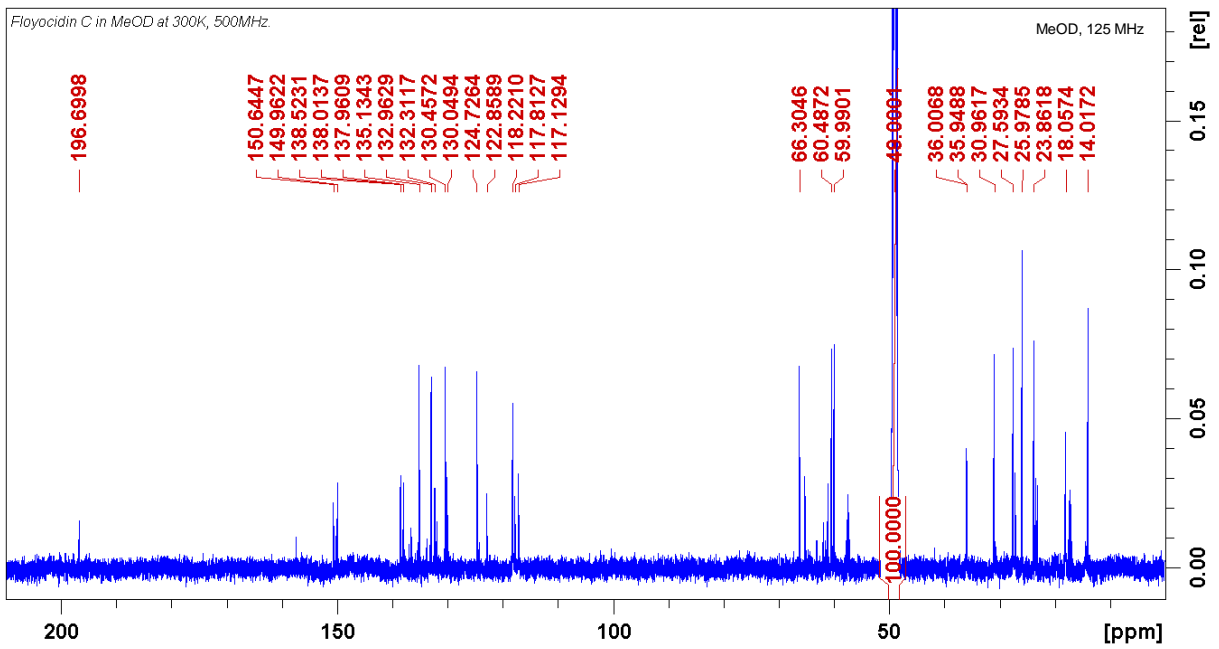
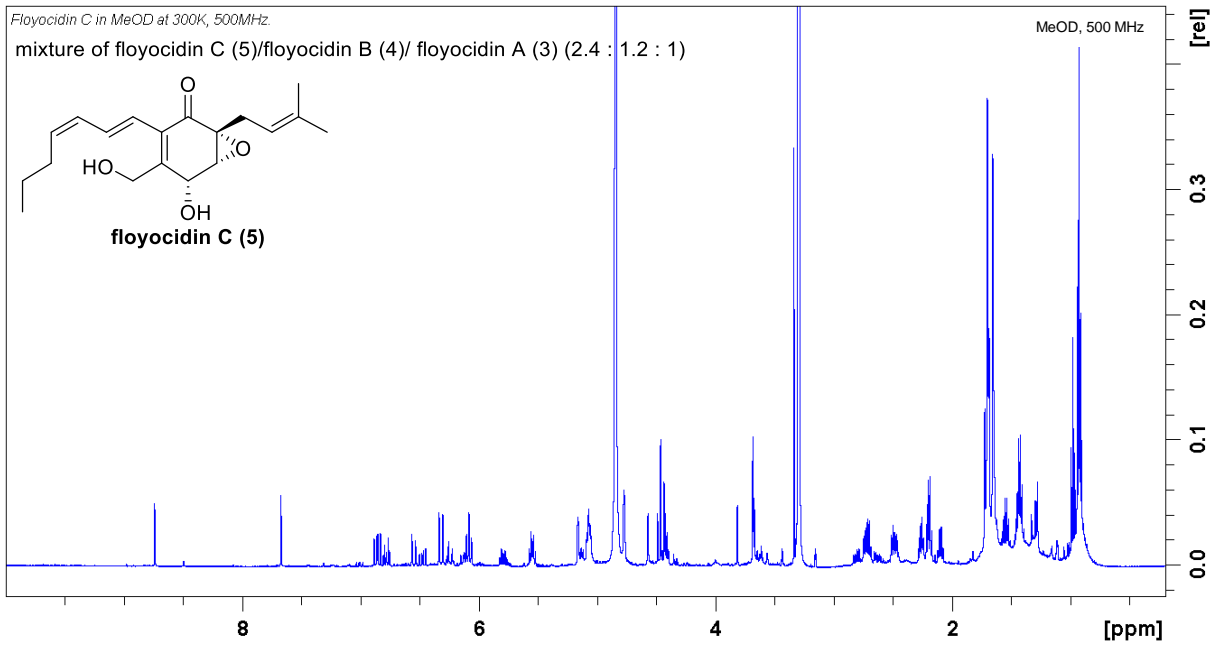
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Empirical formula	C ₂₃ H ₃₇ Br Cl N O ₃ Si	
Formula weight	518.98	
Temperature	100(2) K	
Wavelength	0.71073 Å	
Crystal system	Orthorhombic	
Space group	C222 ₁	
Unit cell dimensions	a = 11.0712(5) Å	α = 90°.
	b = 20.0242(9) Å	β = 90°.
	c = 23.2586(11) Å	γ = 90°.
Volume	5156.2(4) Å ³	
Z	8	
Density (calculated)	1.337 Mg/m ³	
Absorption coefficient	1.768 mm ⁻¹	
F(000)	2176	
Crystal size	0.309 x 0.095 x 0.072 mm ³	
Theta range for data collection	2.034 to 28.279°.	
Index ranges	-14 ≤ h ≤ 14, -26 ≤ k ≤ 26, -31 ≤ l ≤ 31	
Reflections collected	178371	
Independent reflections	6417 [R(int) = 0.0474]	
Completeness to theta = 25.242°	100.0%	
Absorption correction	Semi-empirical from equivalents	
Refinement method	Full-matrix least-squares on F ²	
Data / restraints / parameters	6417 / 1397 / 529	
Goodness-of-fit on F ²	1.055	
Final R indices [I > 2σ(I)]	R ₁ = 0.0324, wR ₂ = 0.0672	
R indices (all data)	R ₁ = 0.0364, wR ₂ = 0.0691	
Absolute structure parameter	0.014(2)	
Extinction coefficient	0.00150(13)	
Largest diff. peak and hole	0.737 and -0.658 e.Å ⁻³	

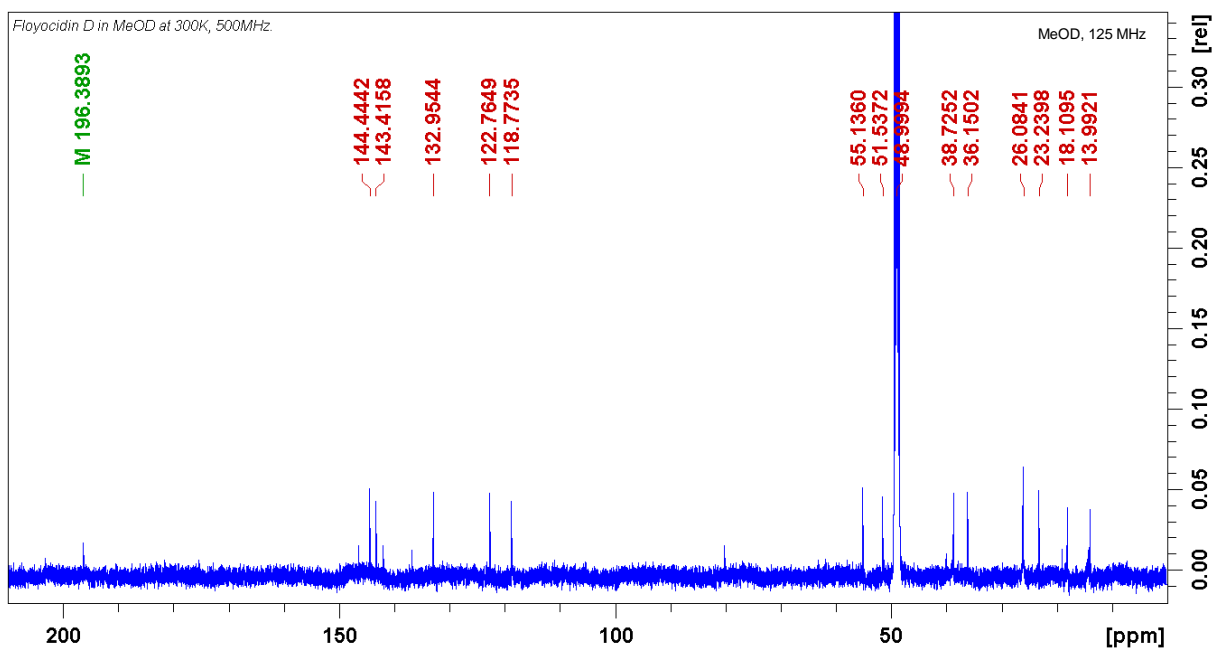
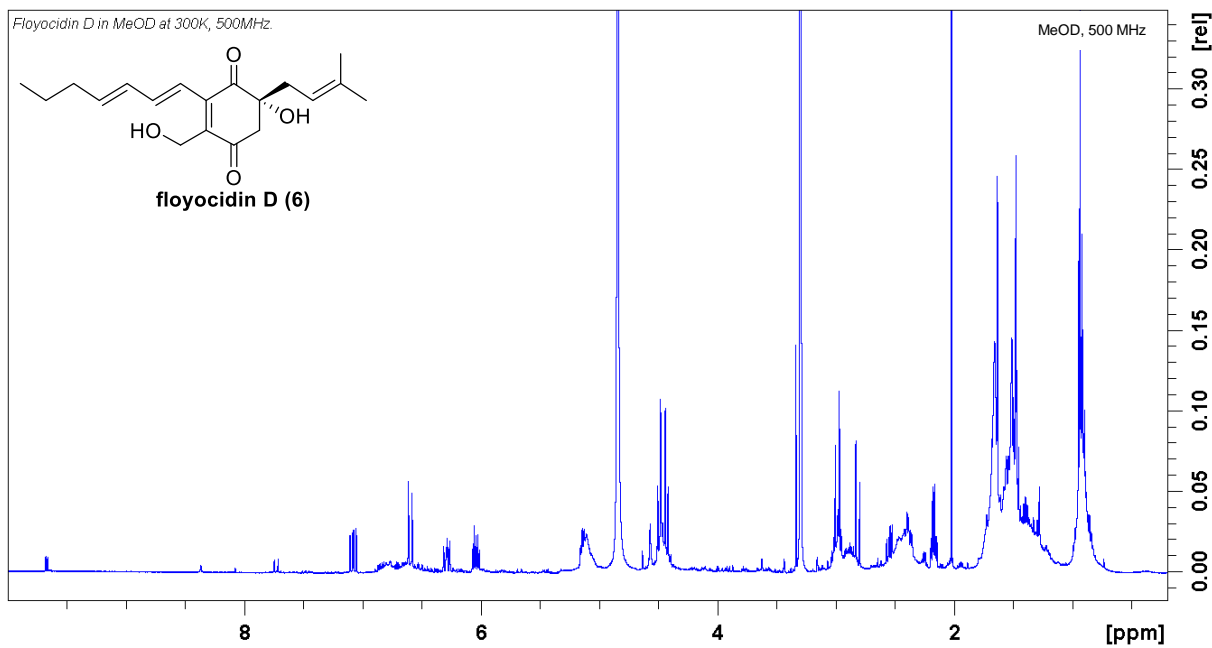
5. NMR spectra

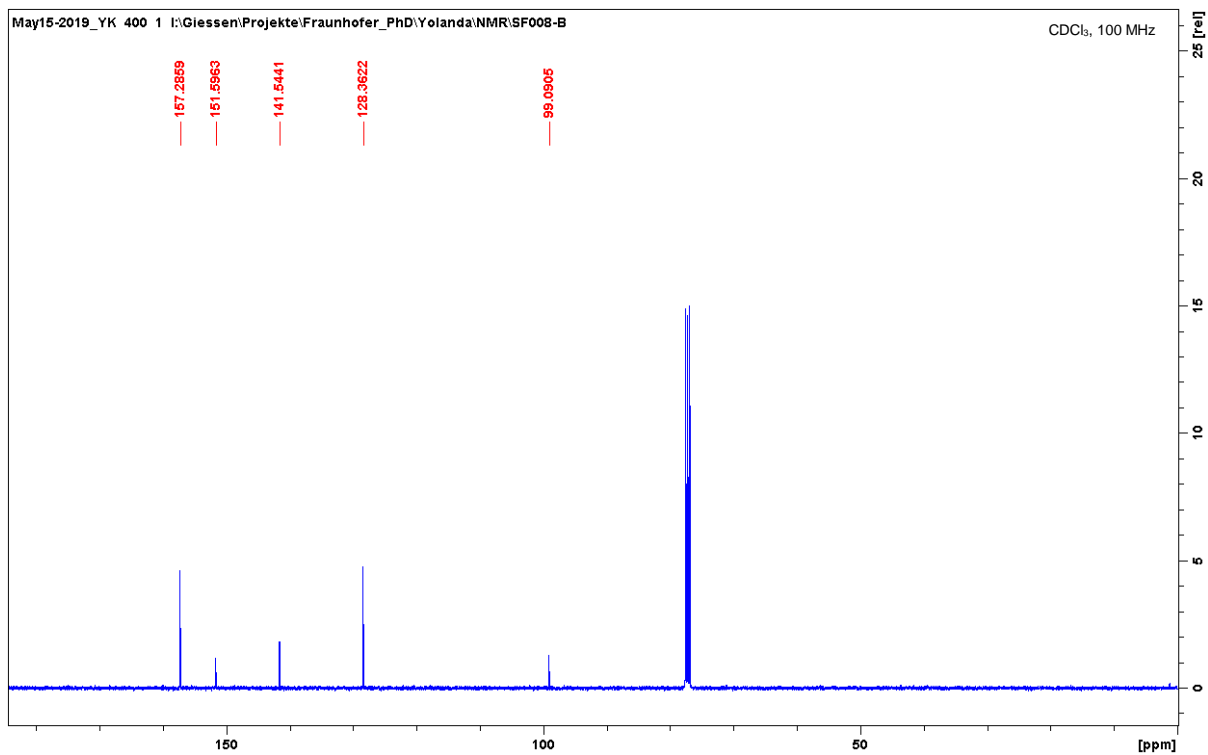
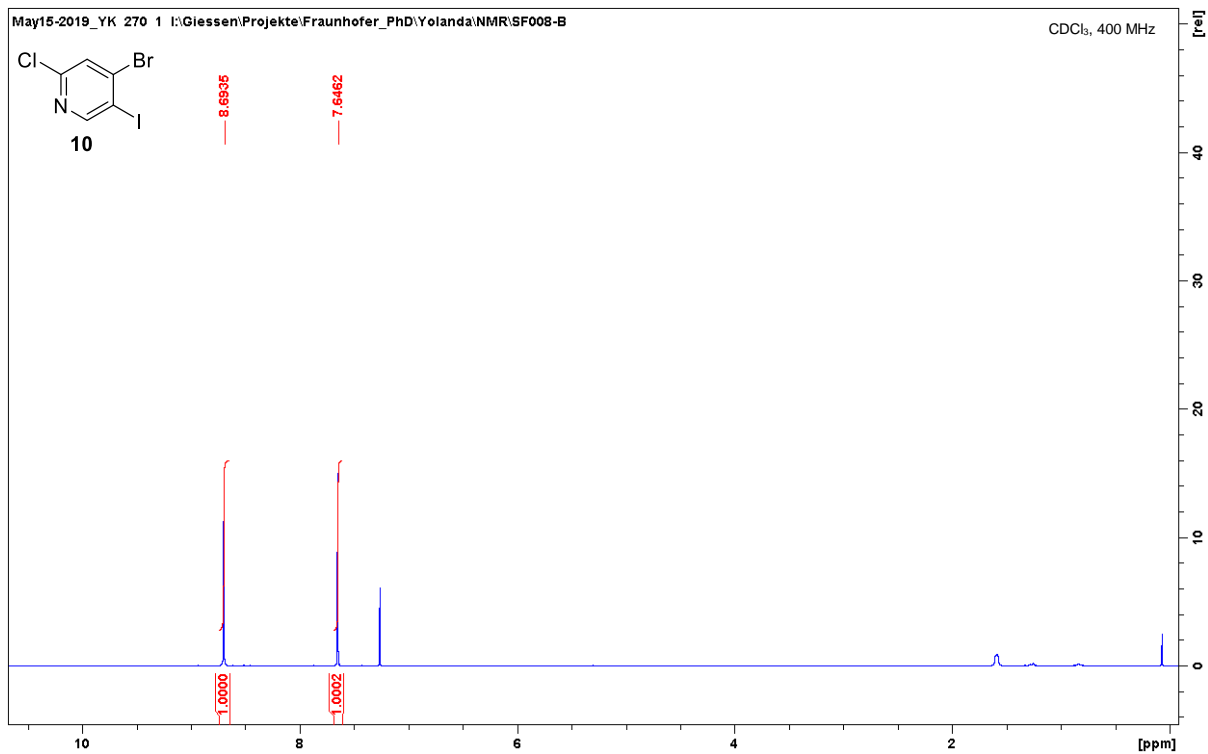


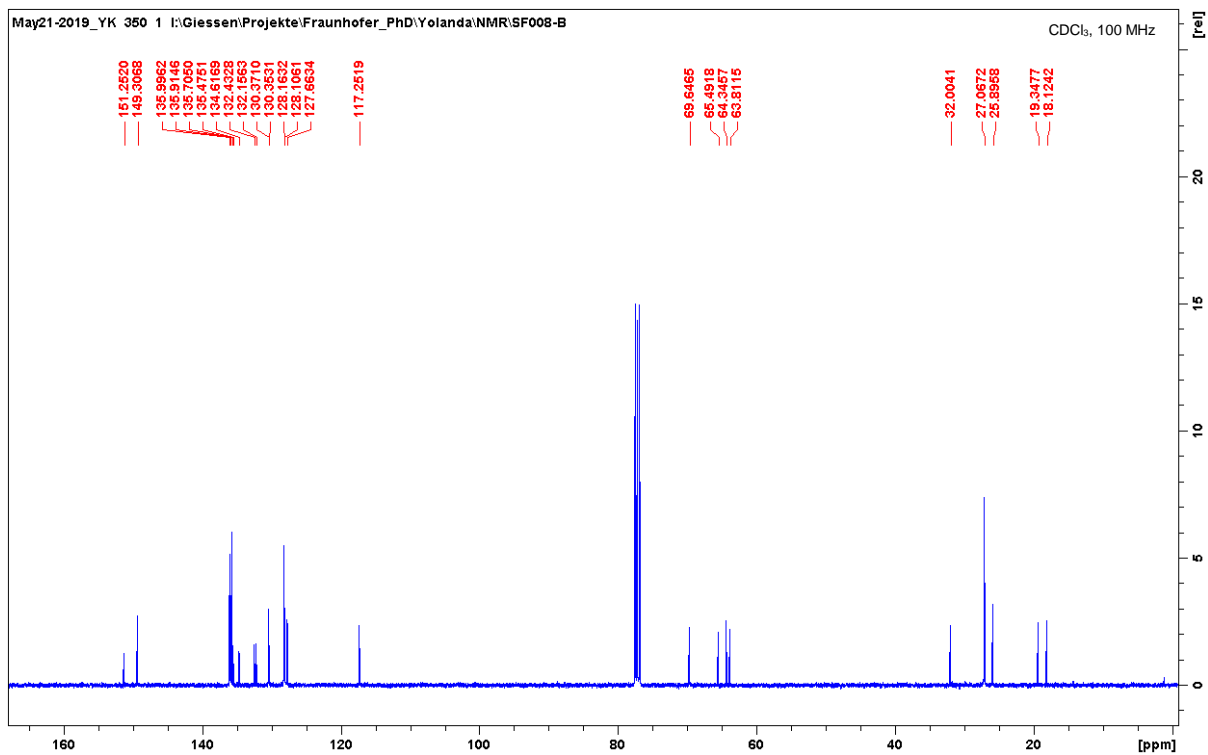
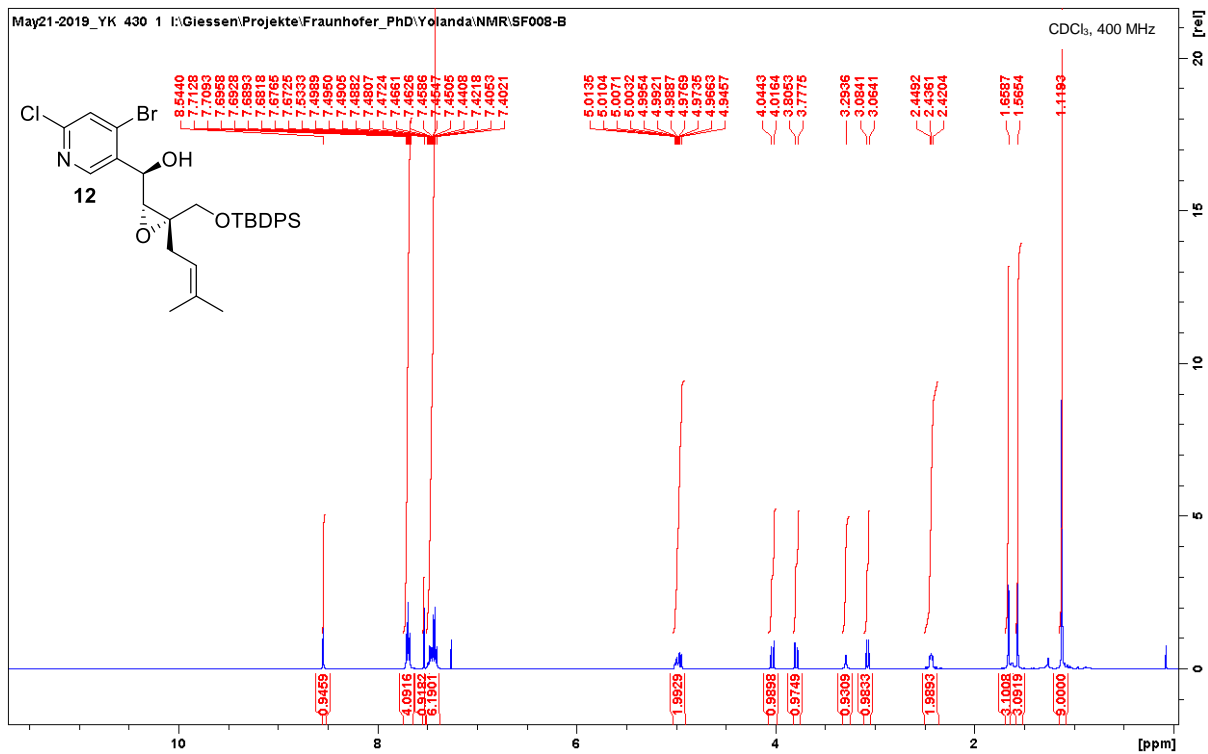


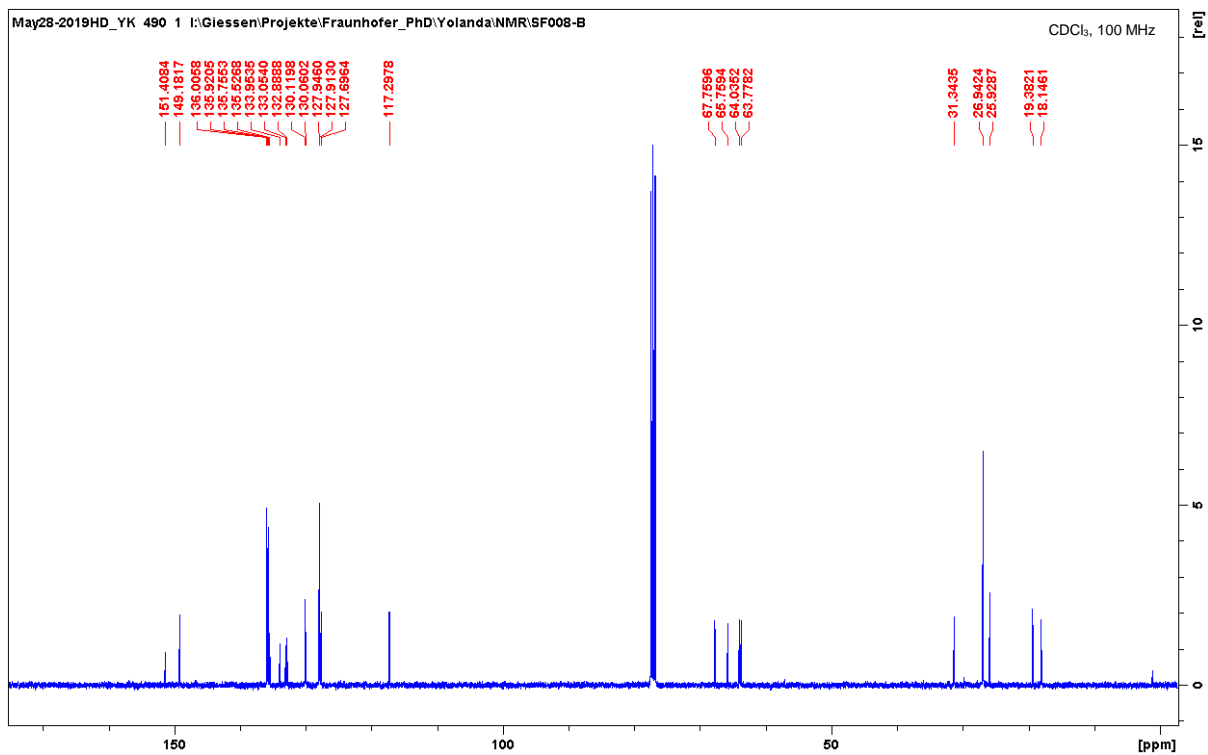
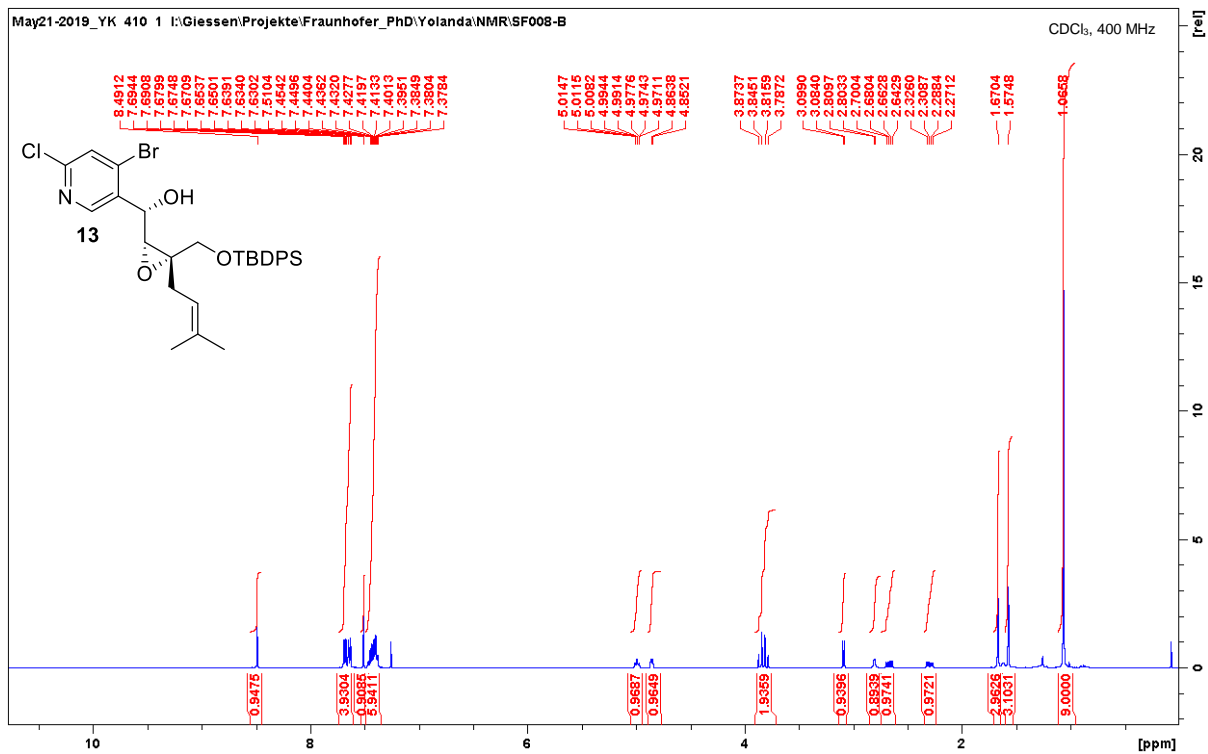


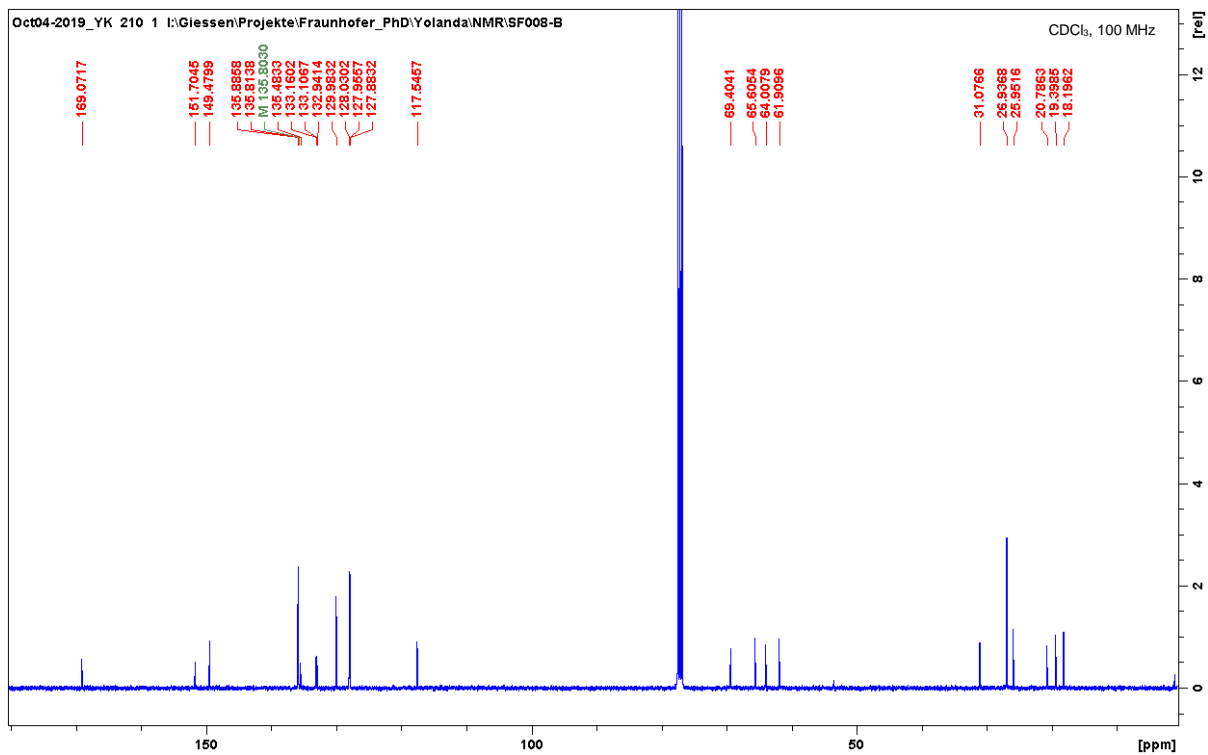
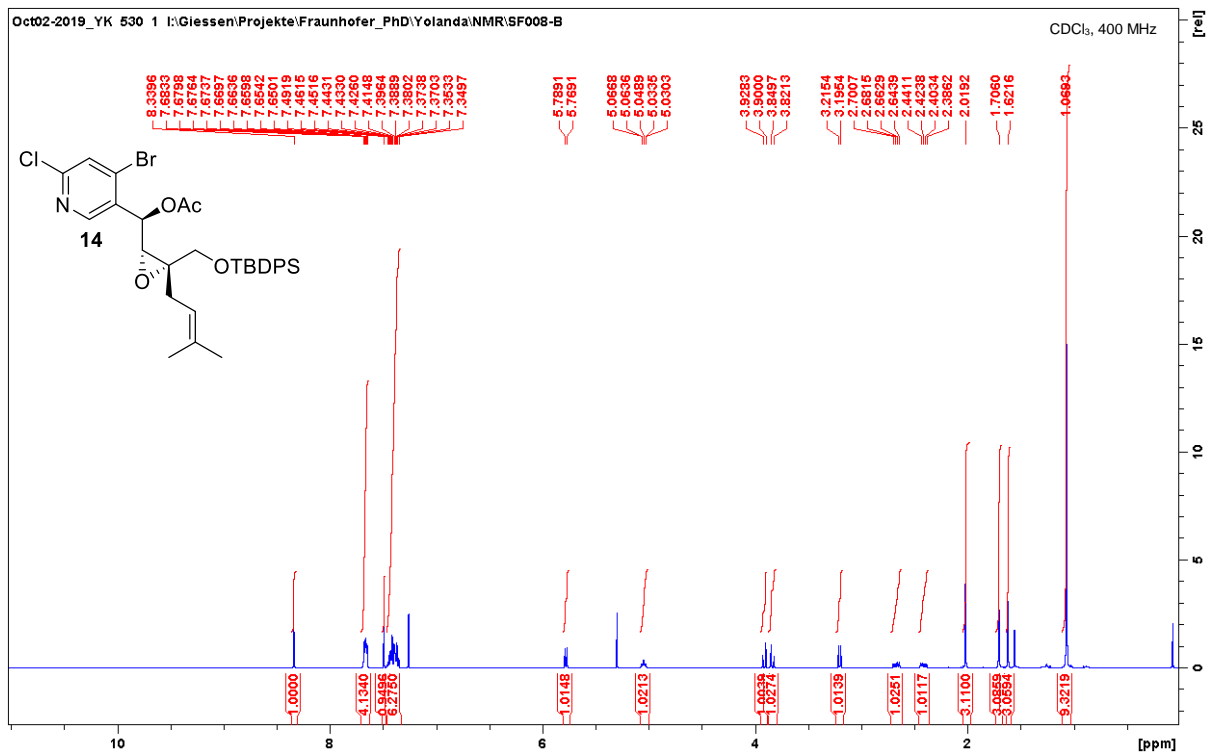


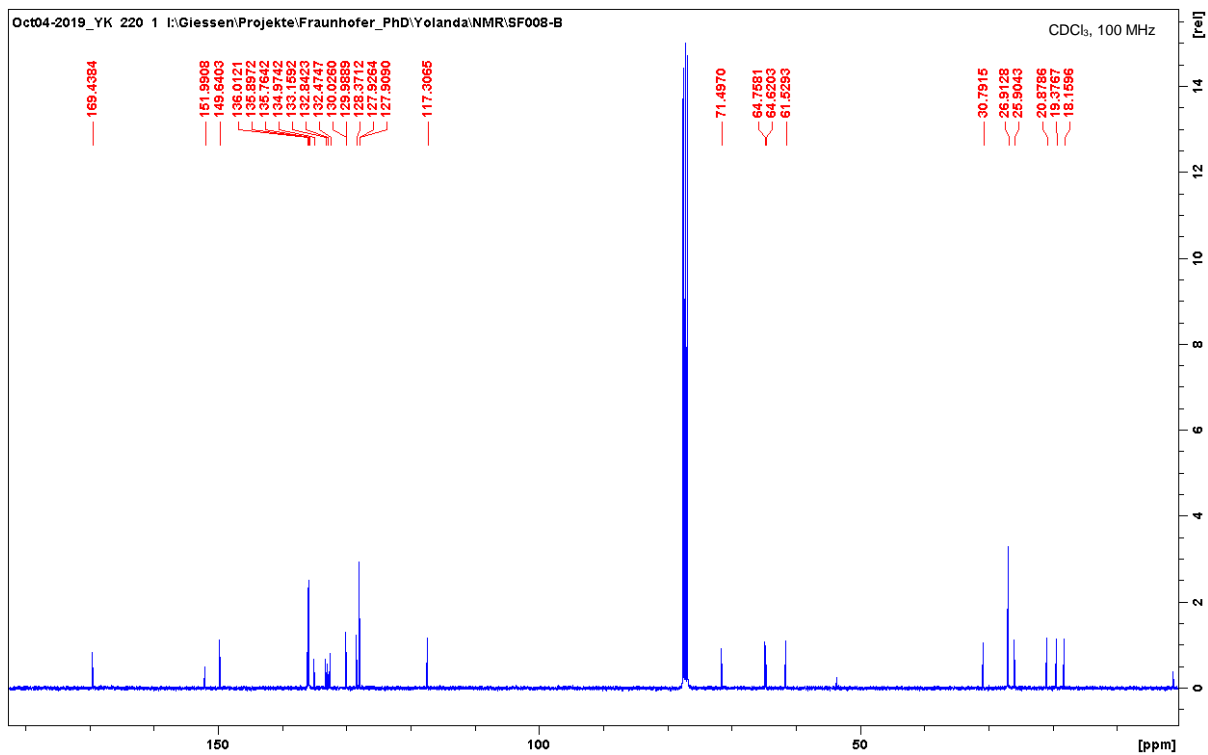
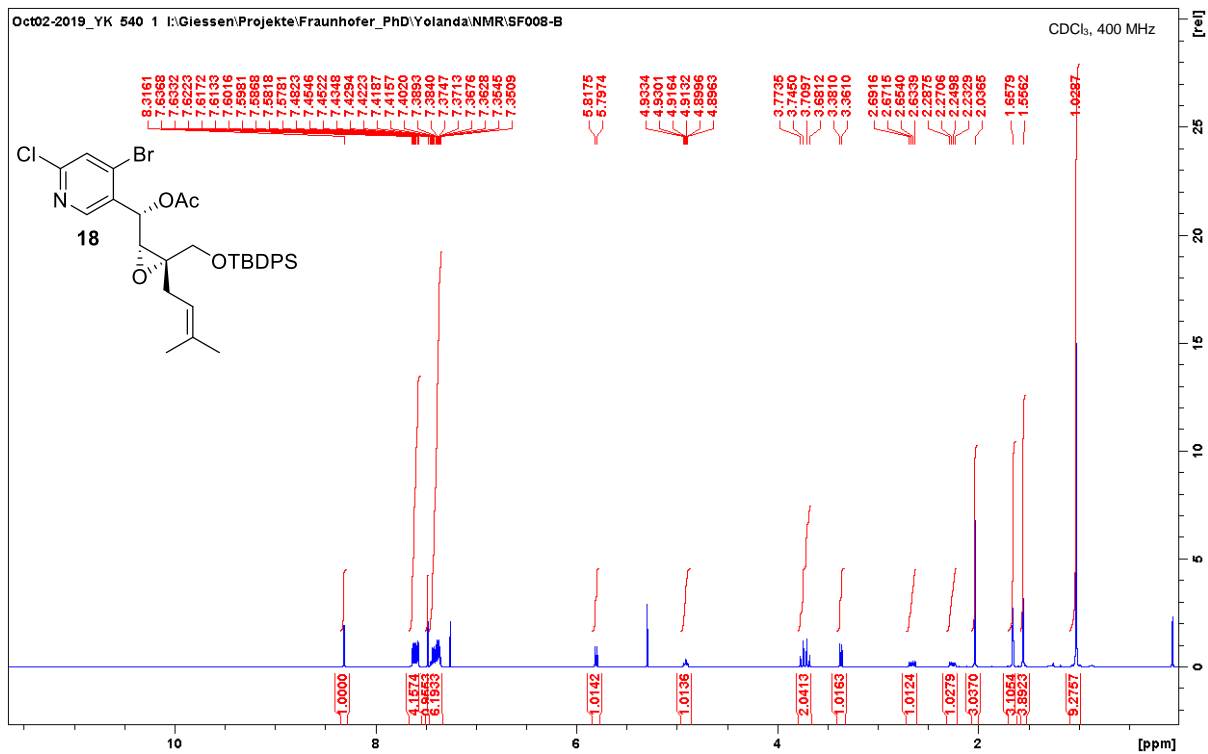


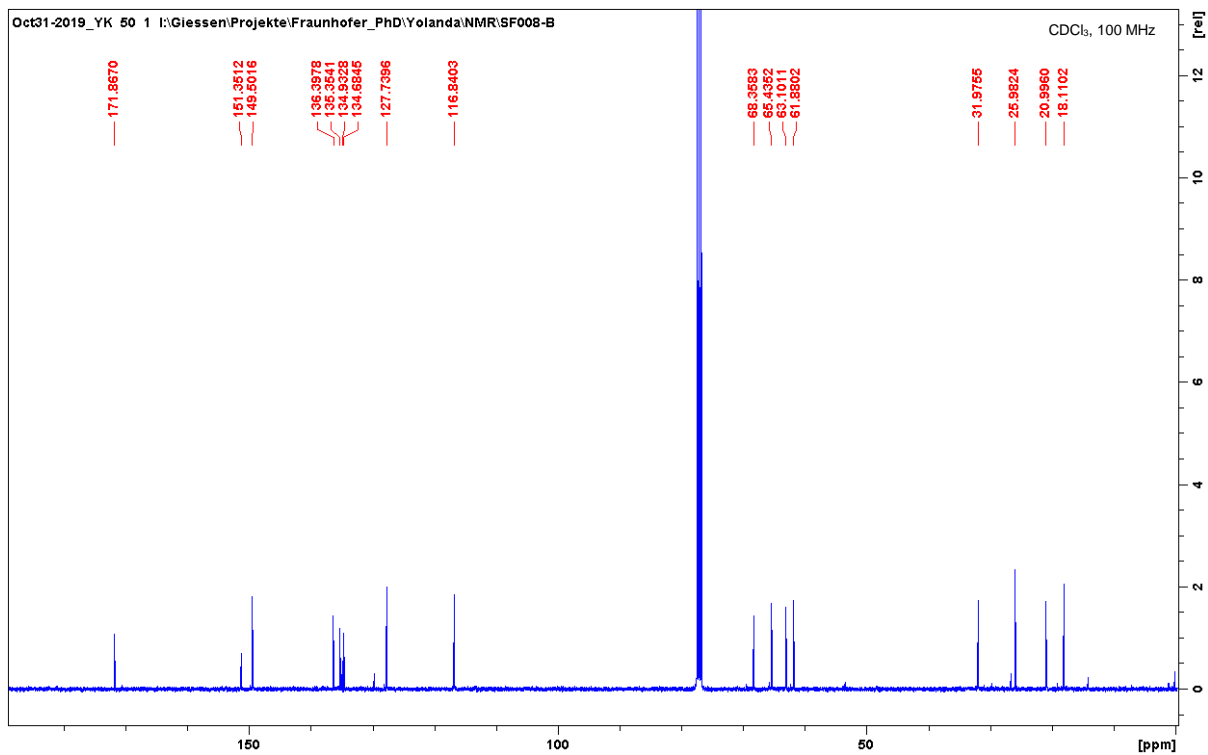
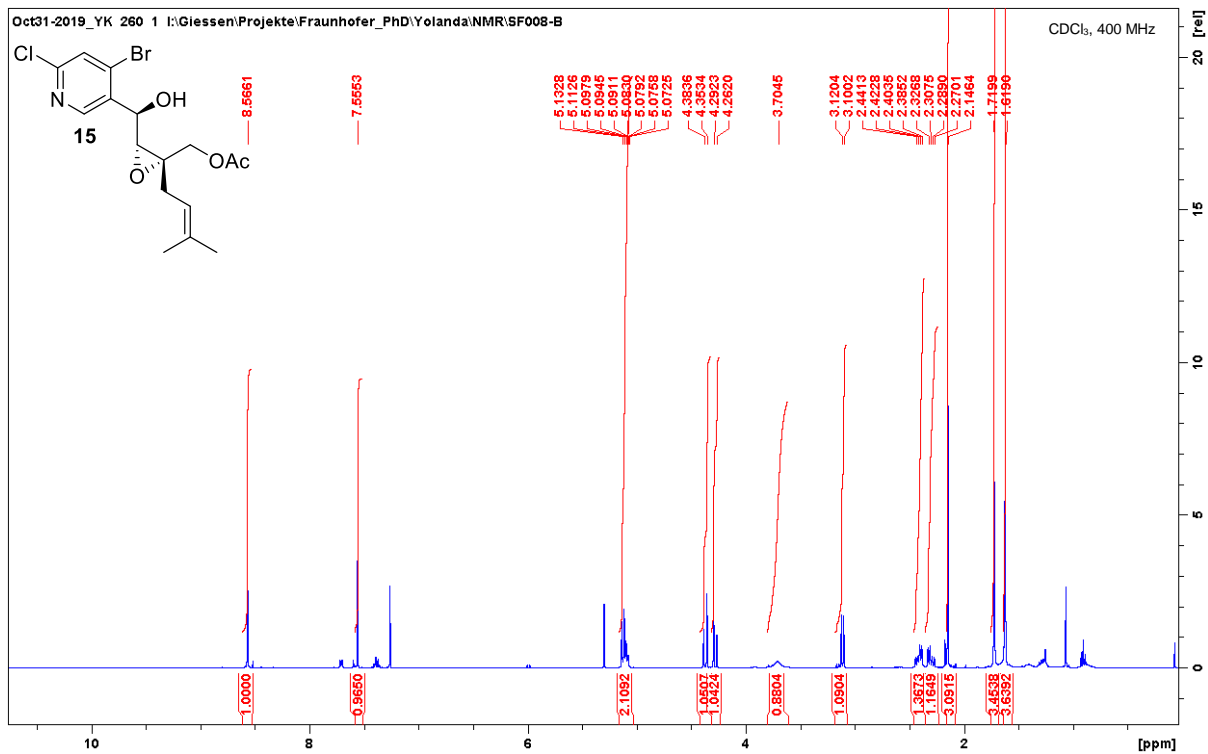


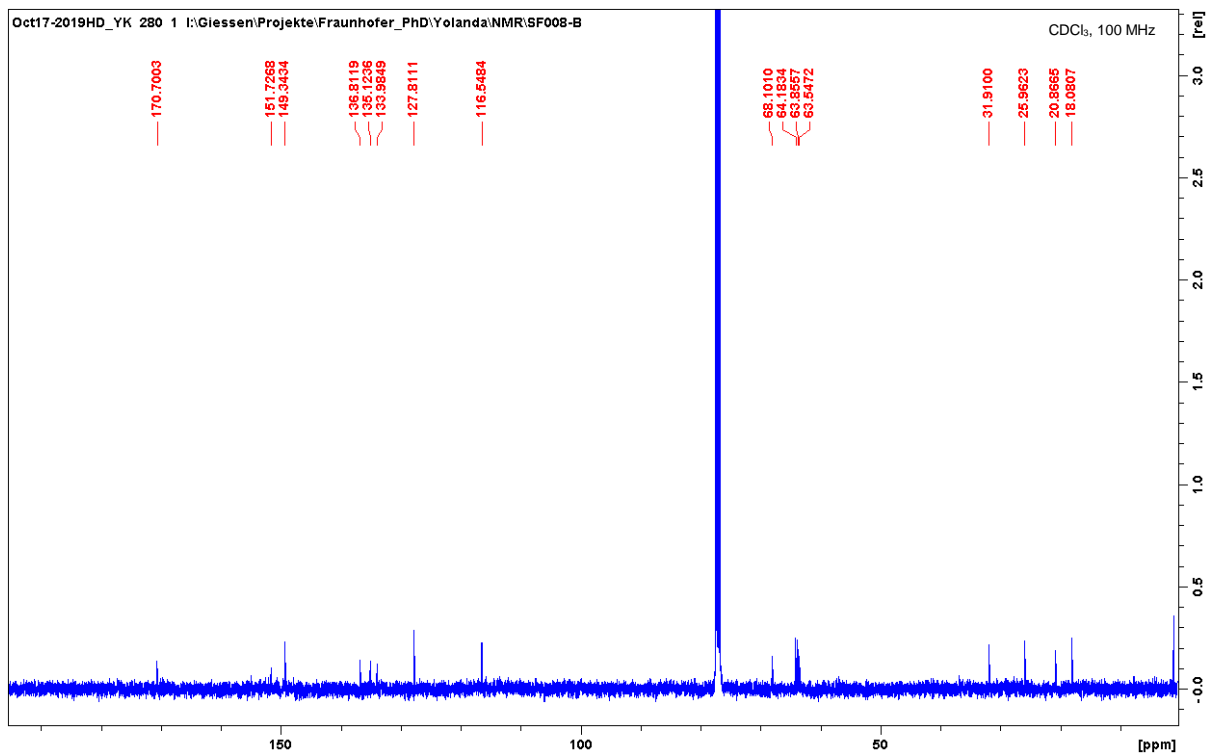
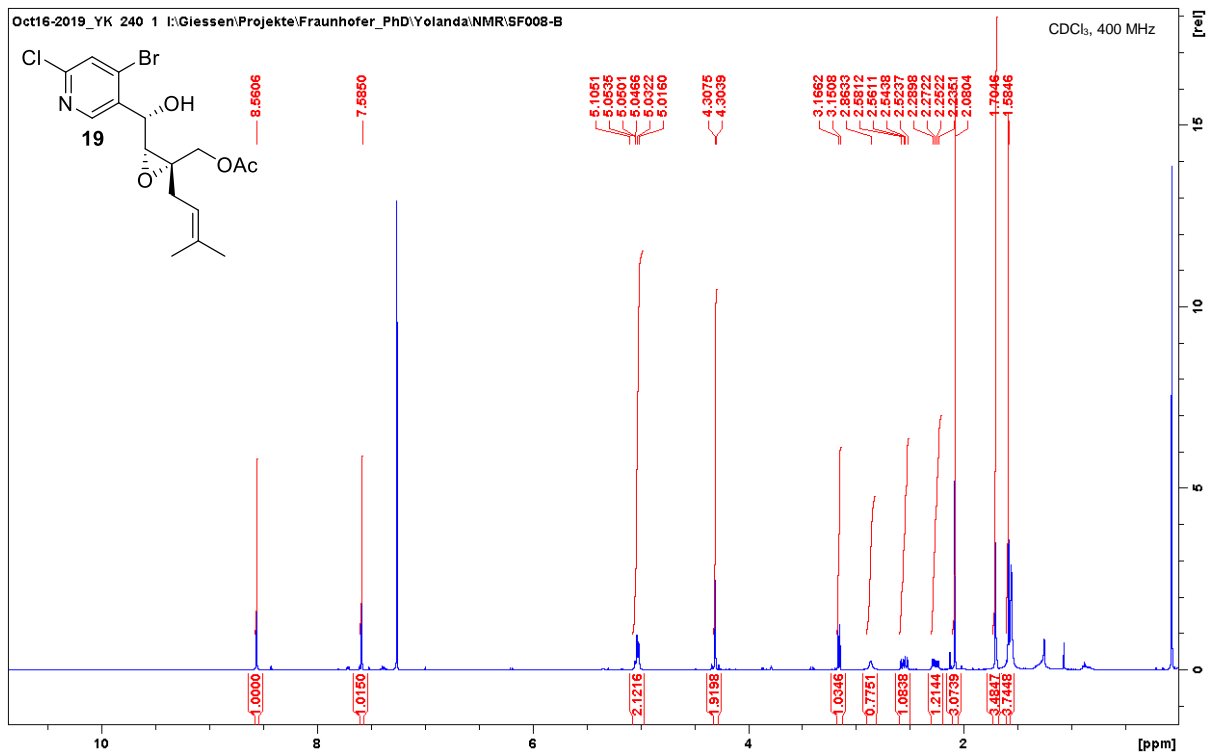


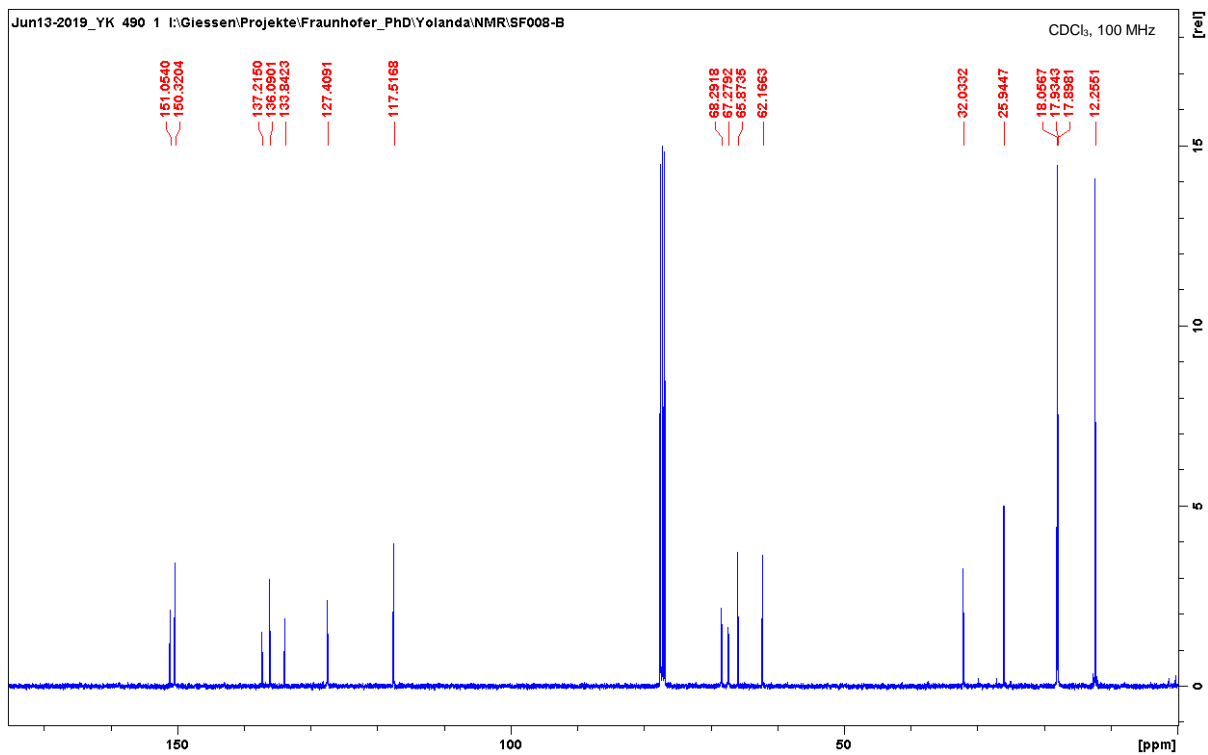
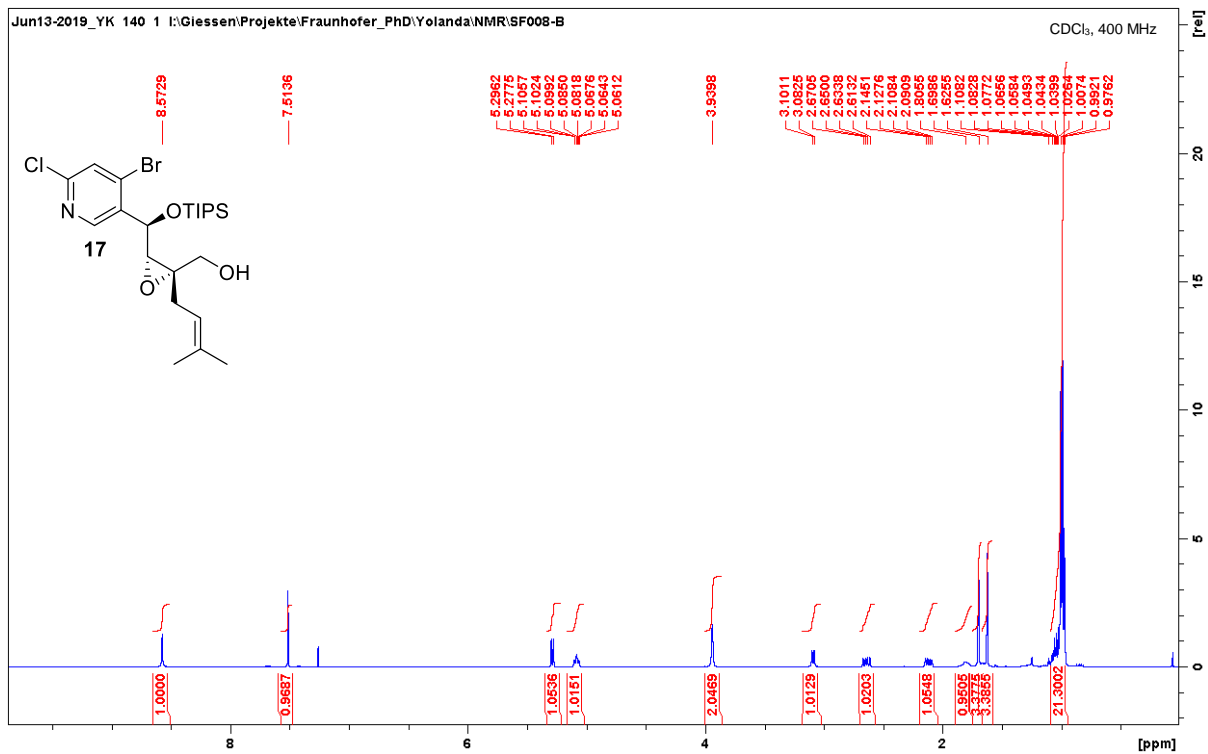


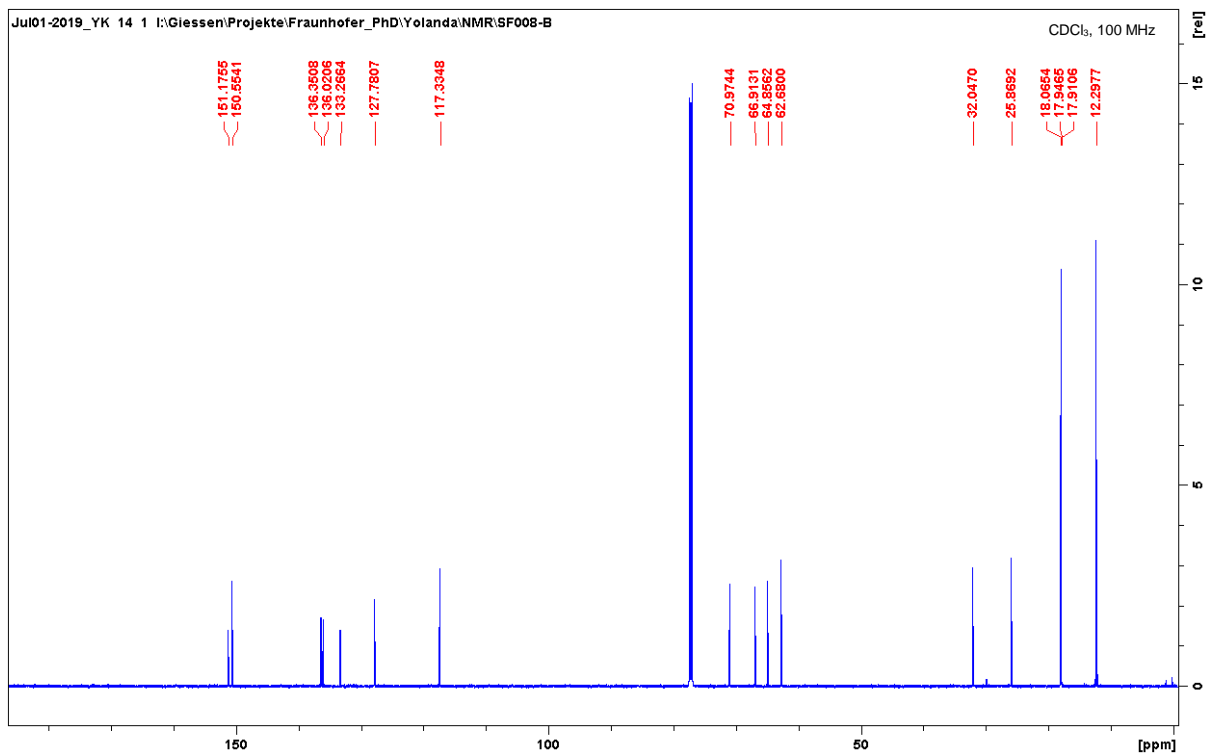
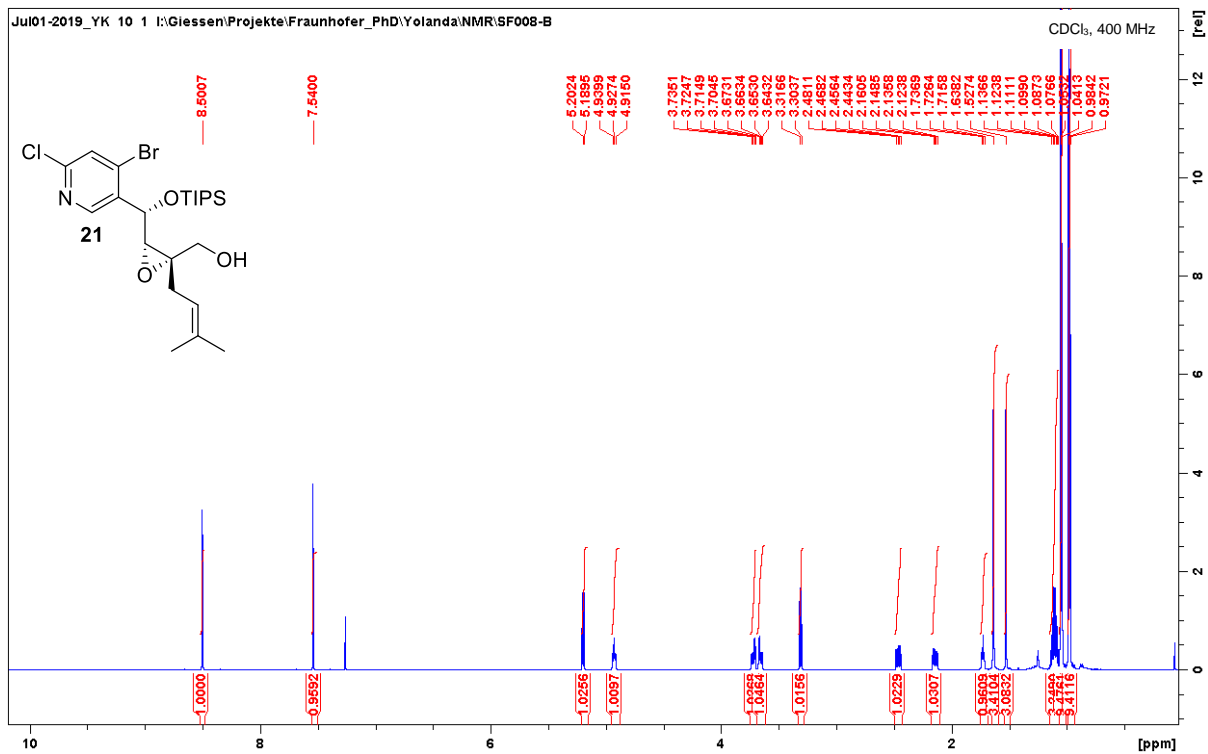


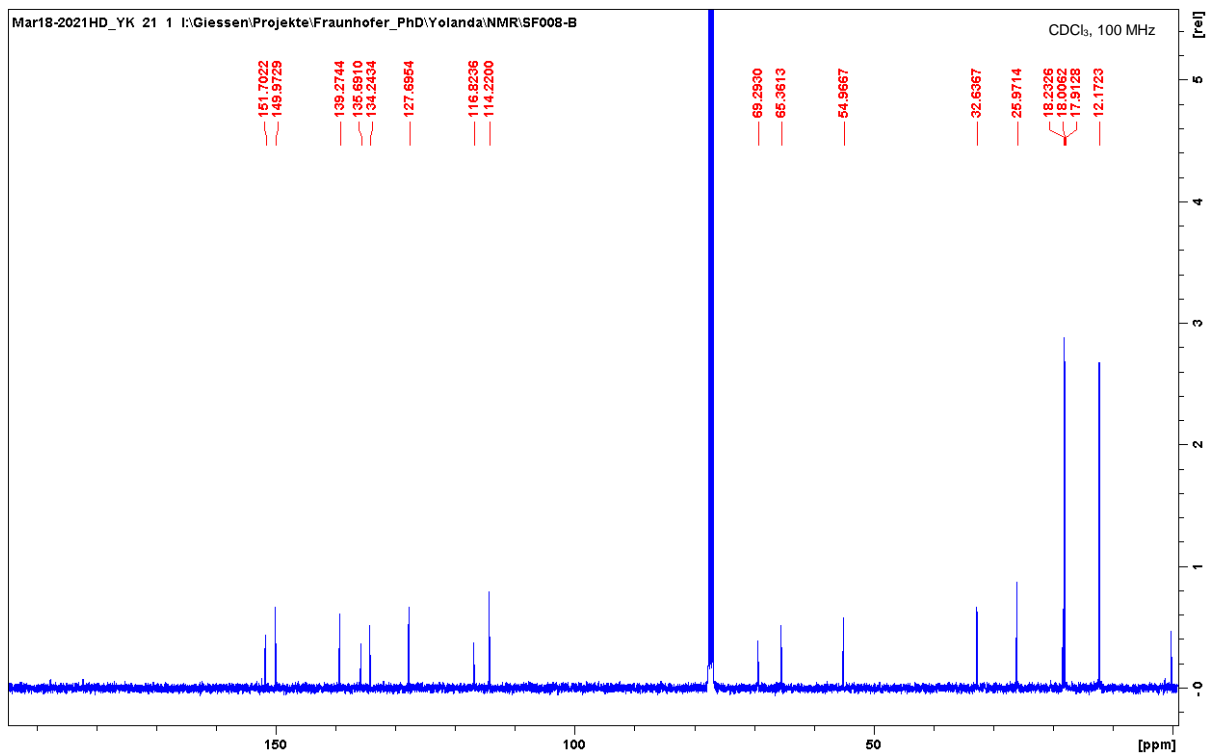
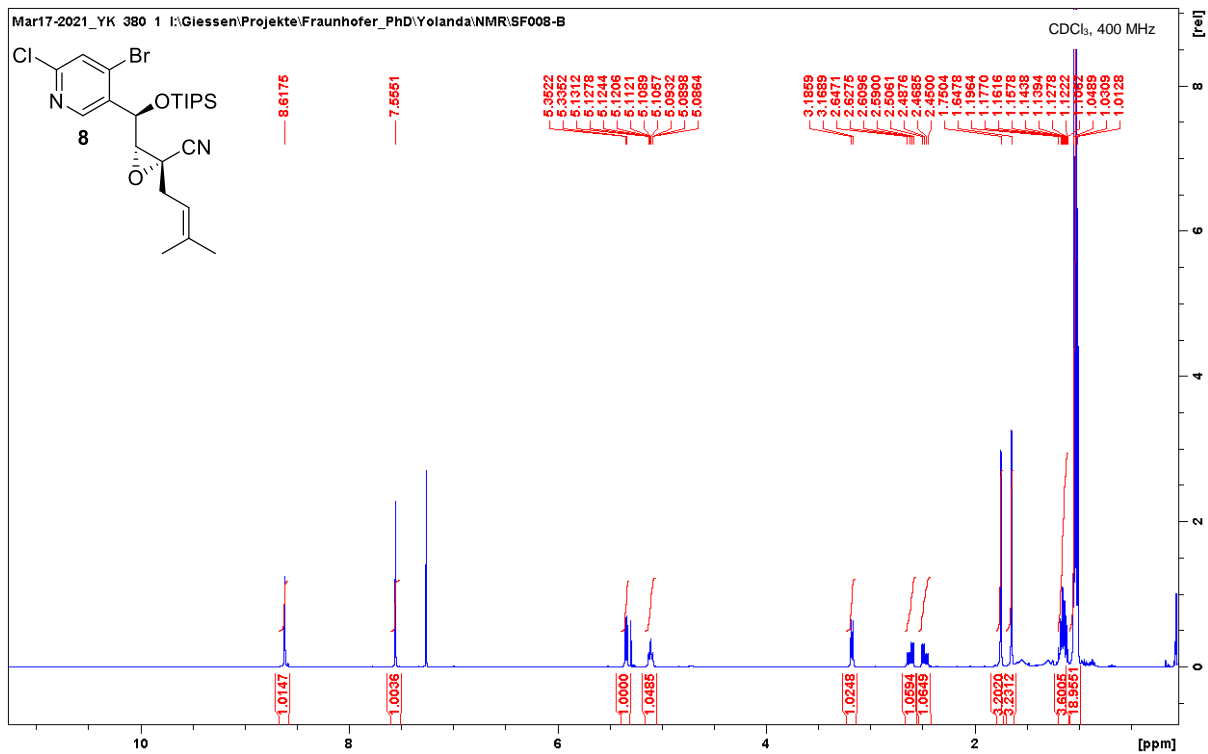


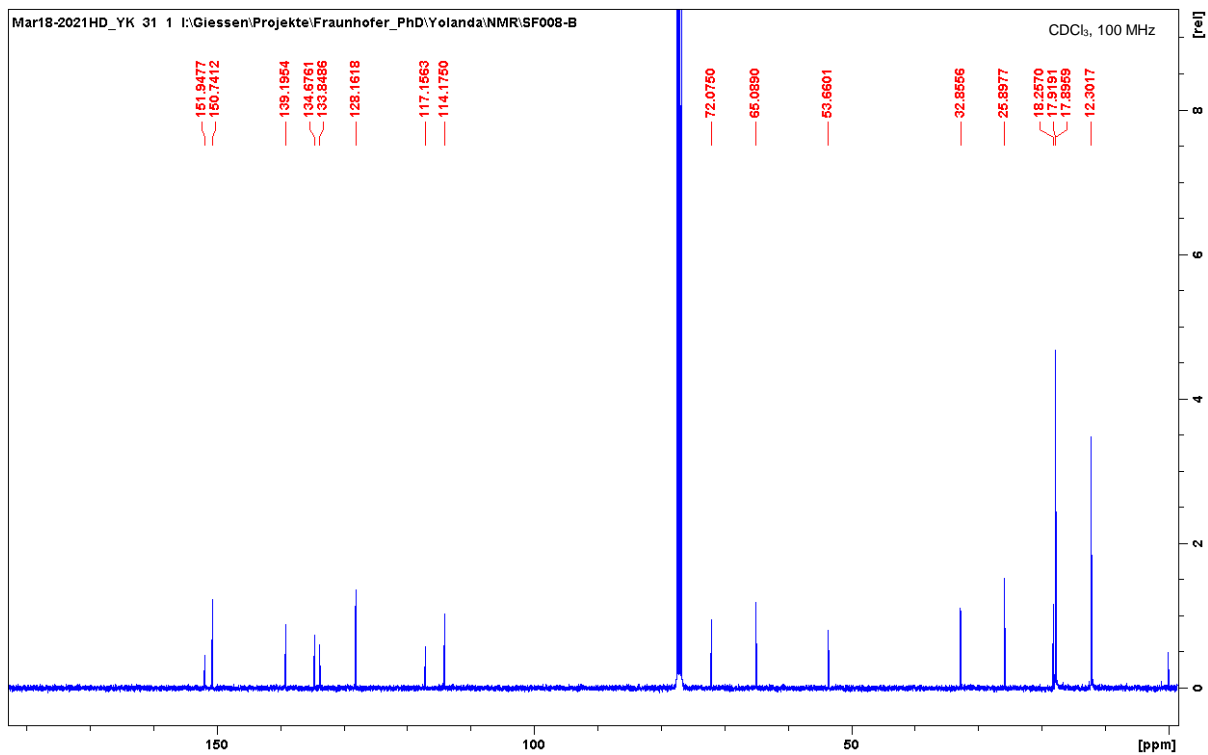
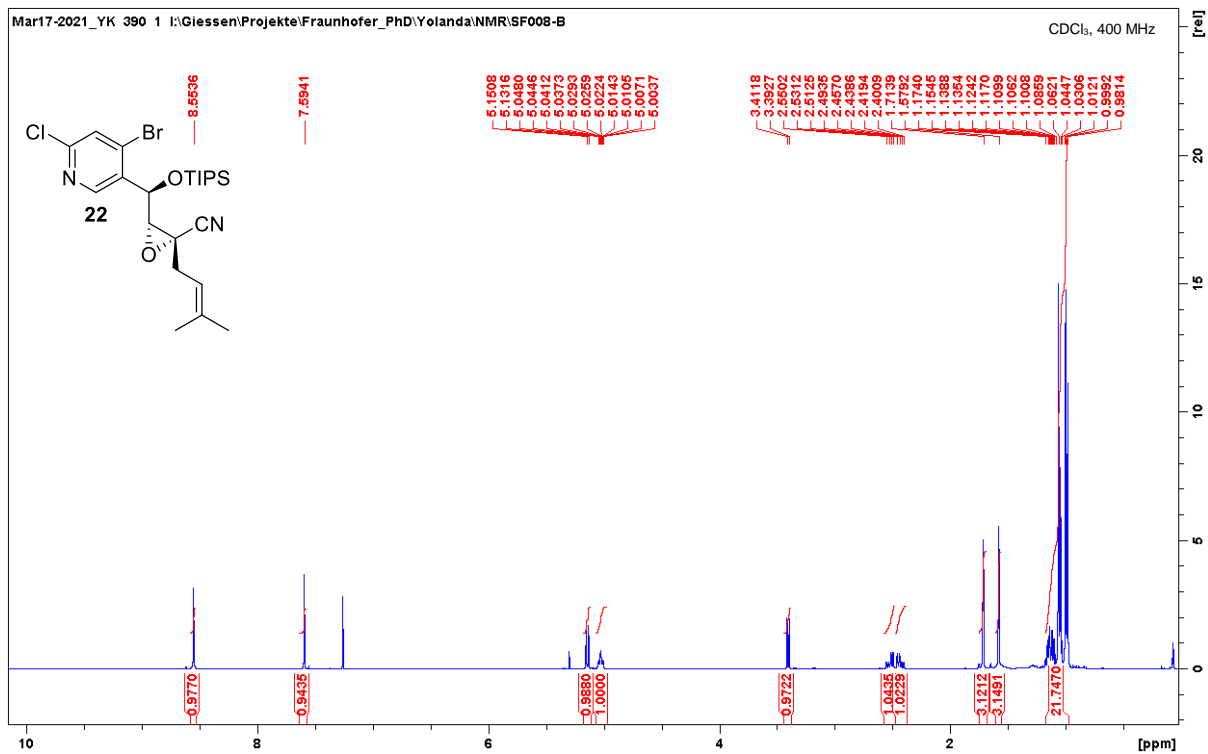


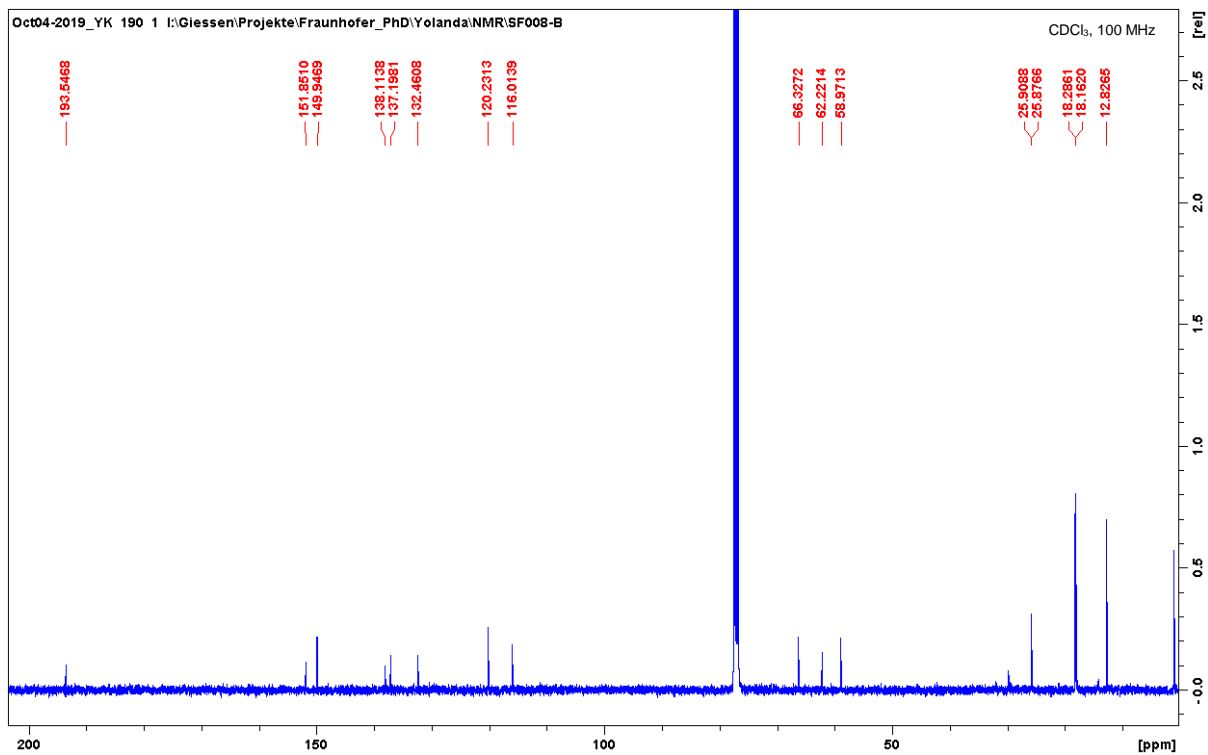
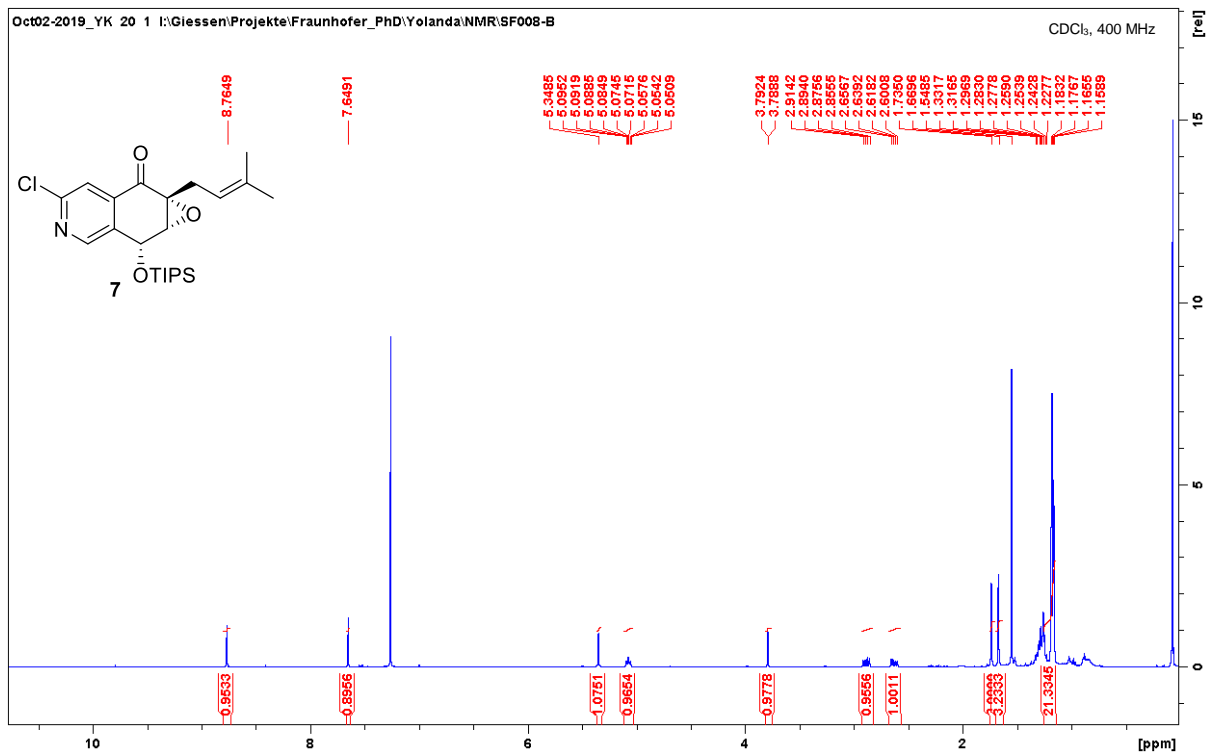


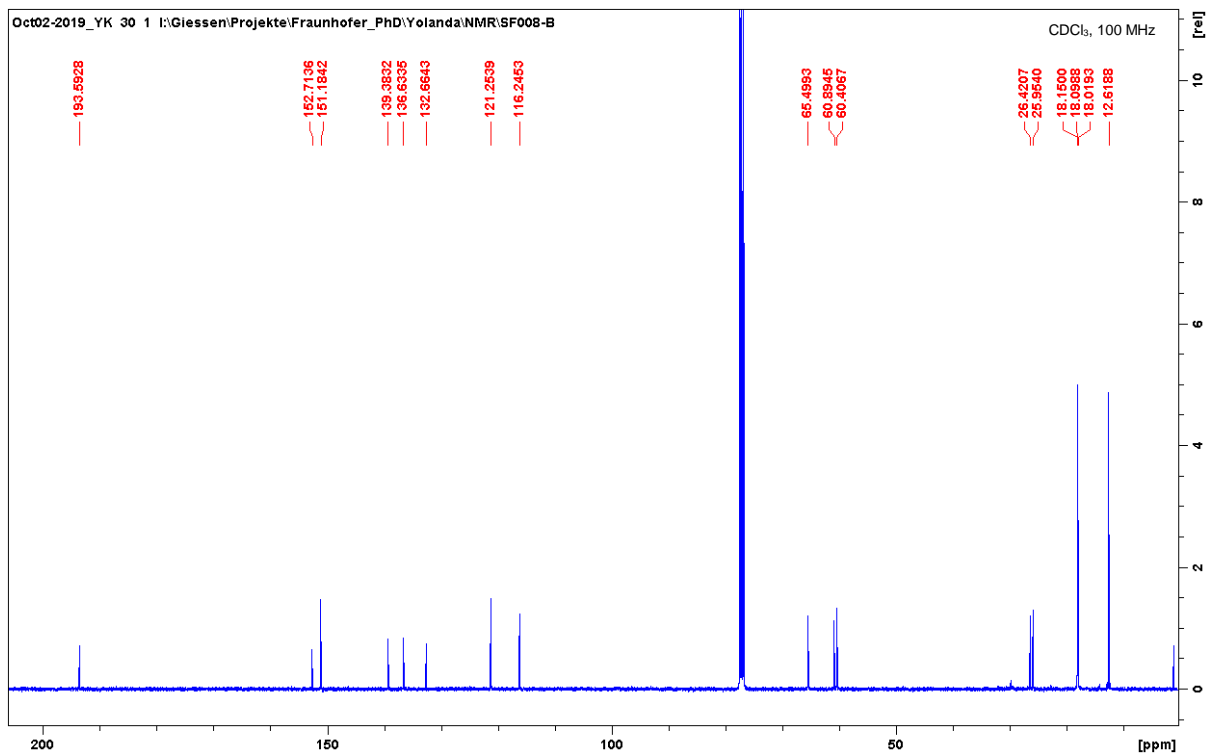
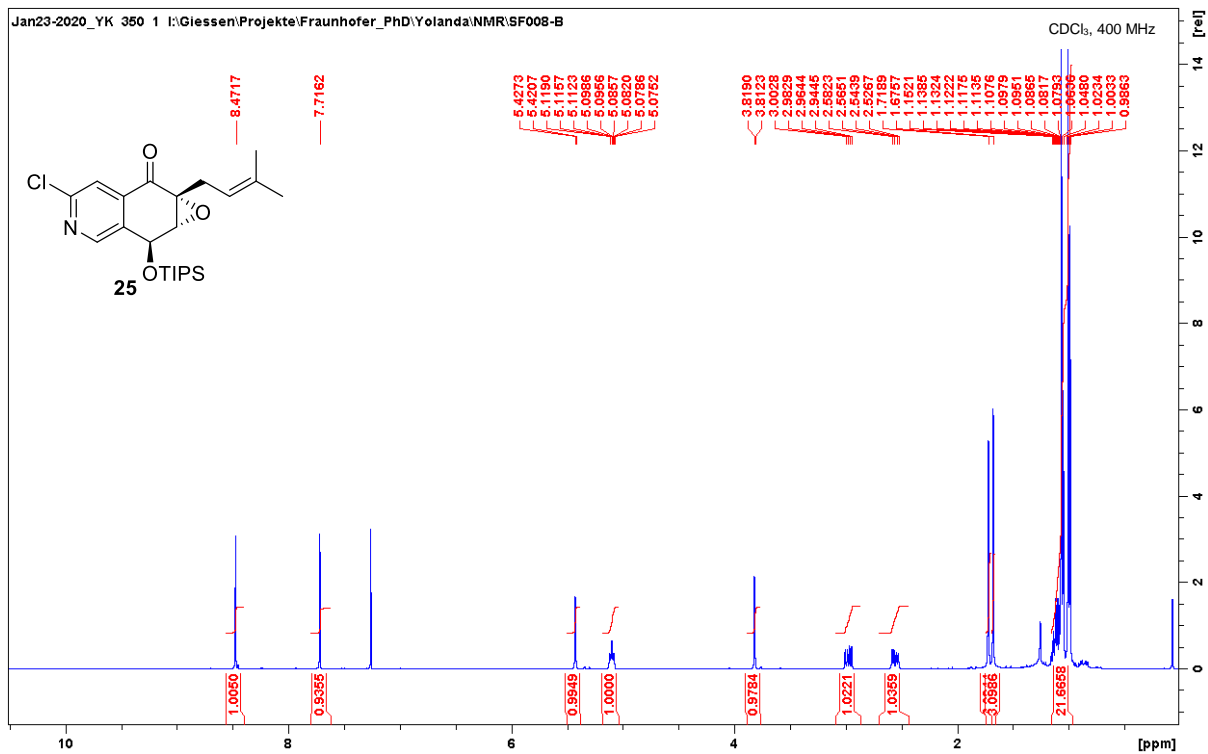


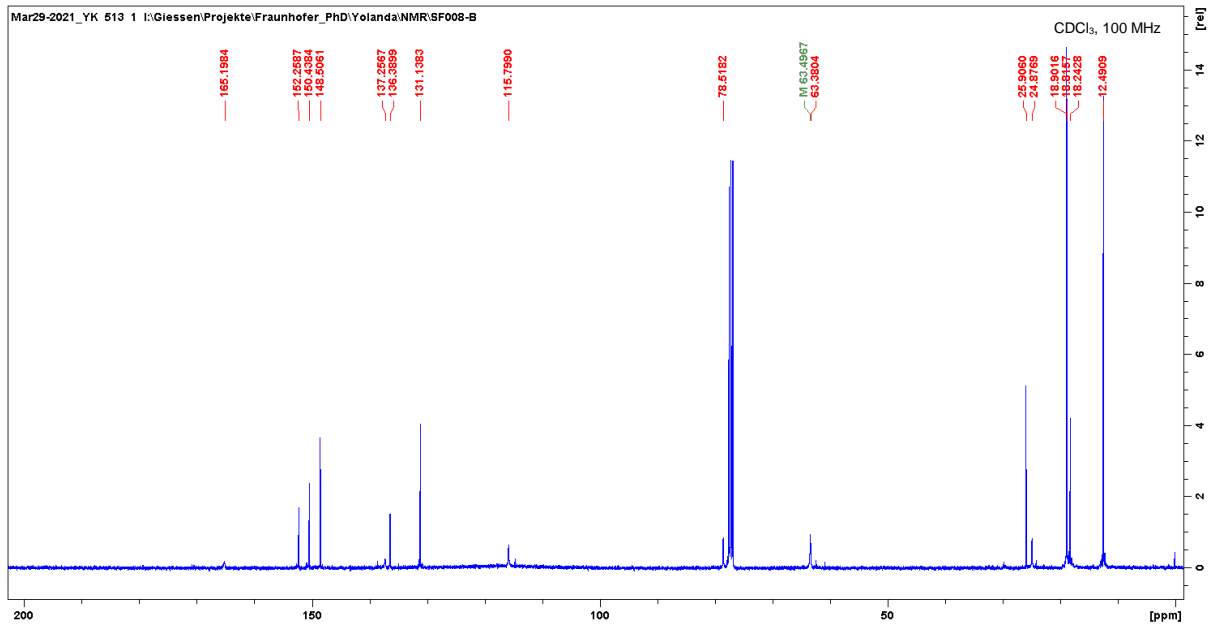
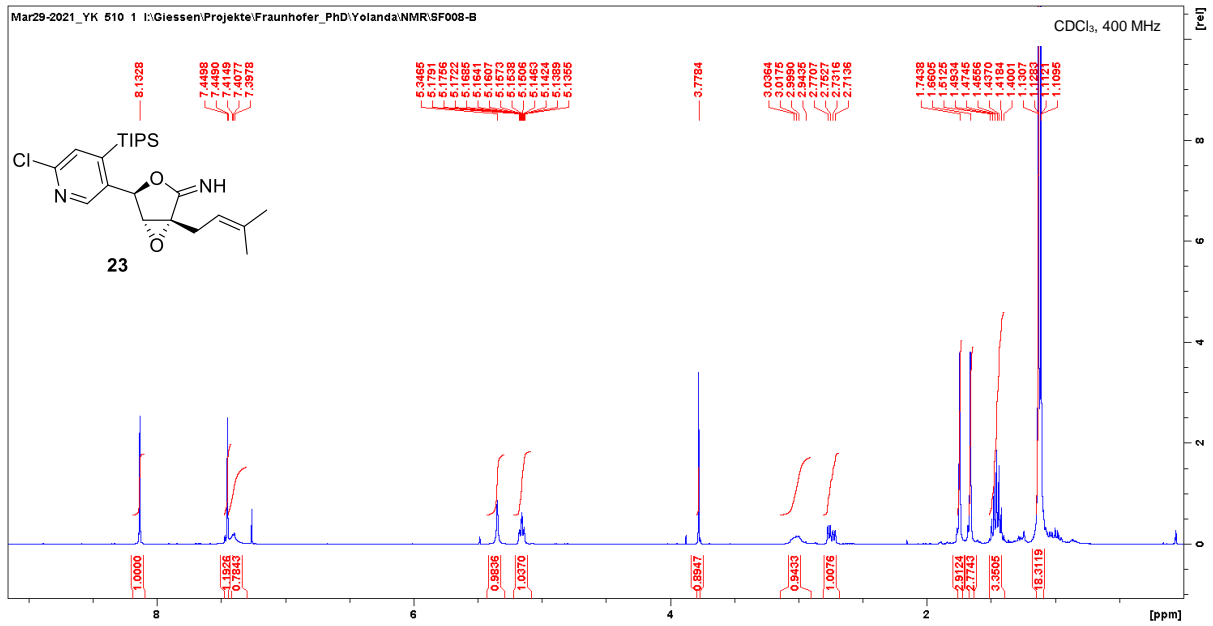


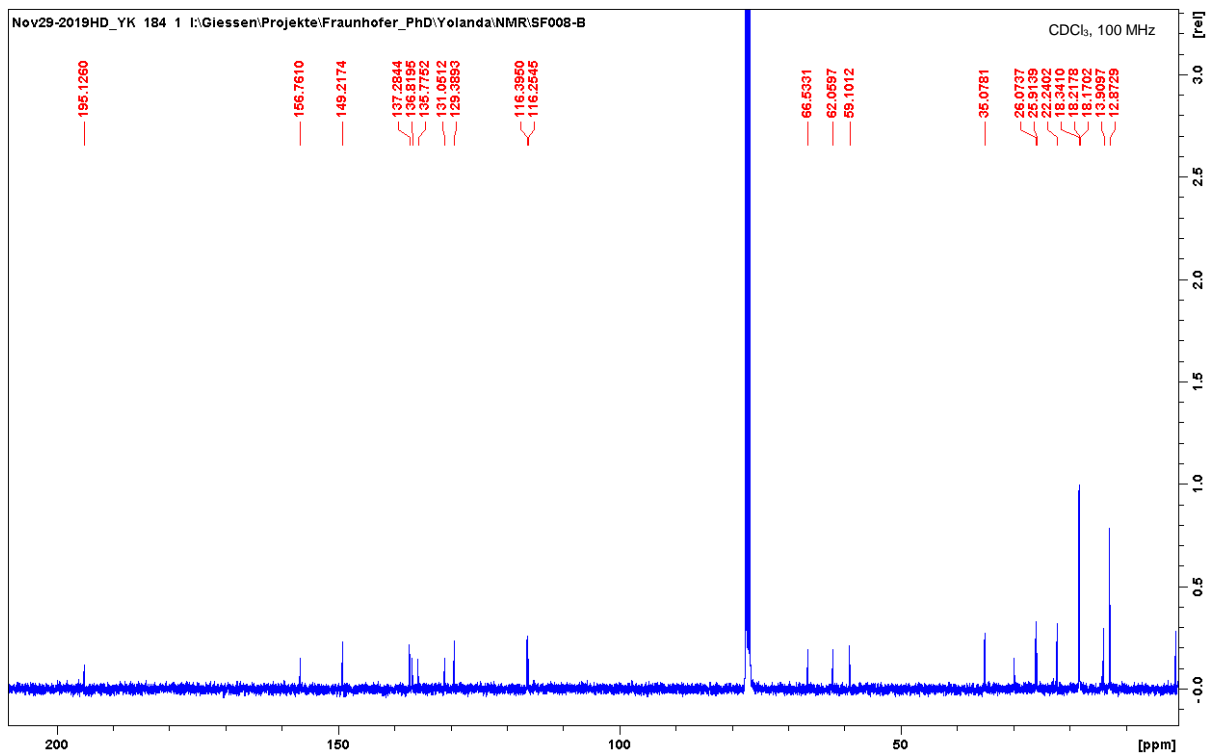
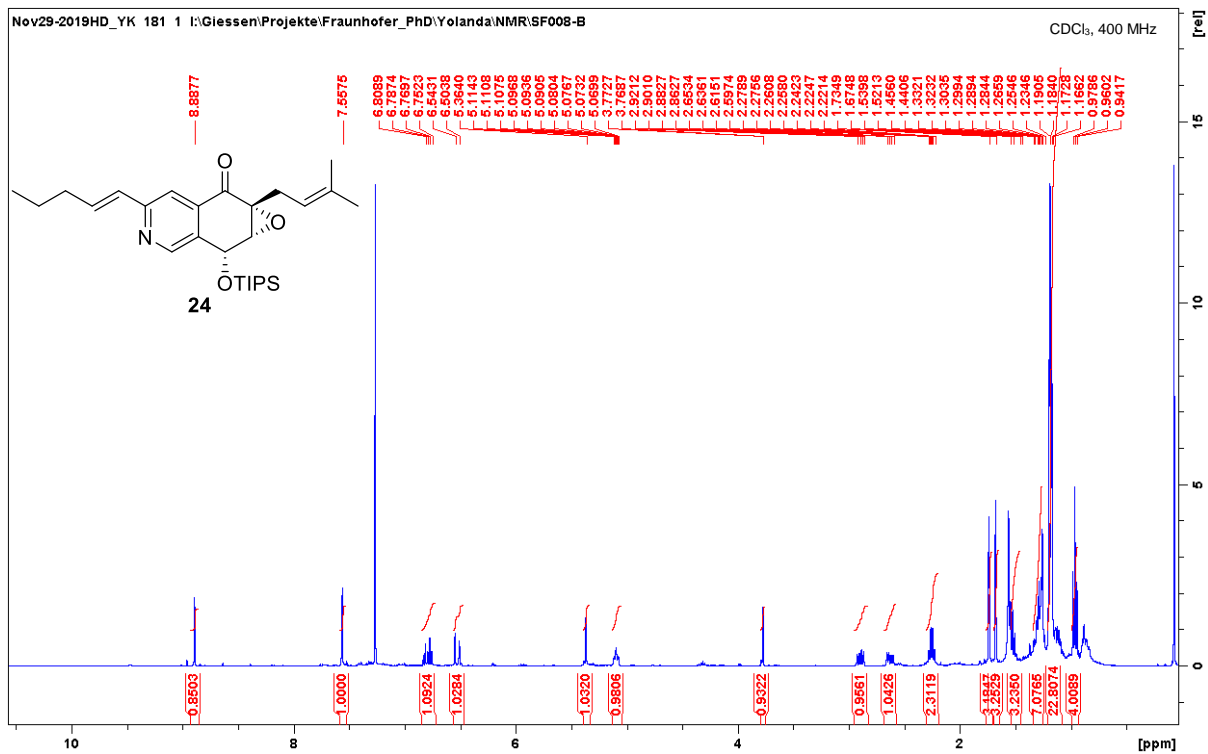


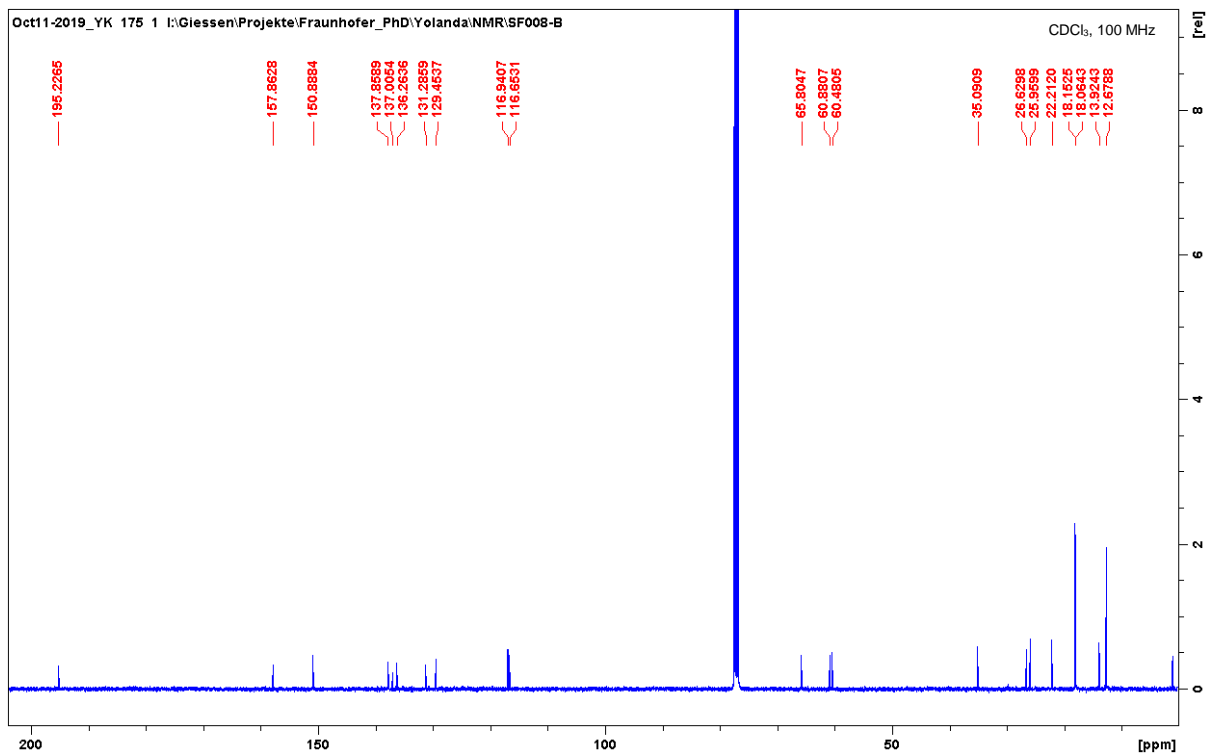
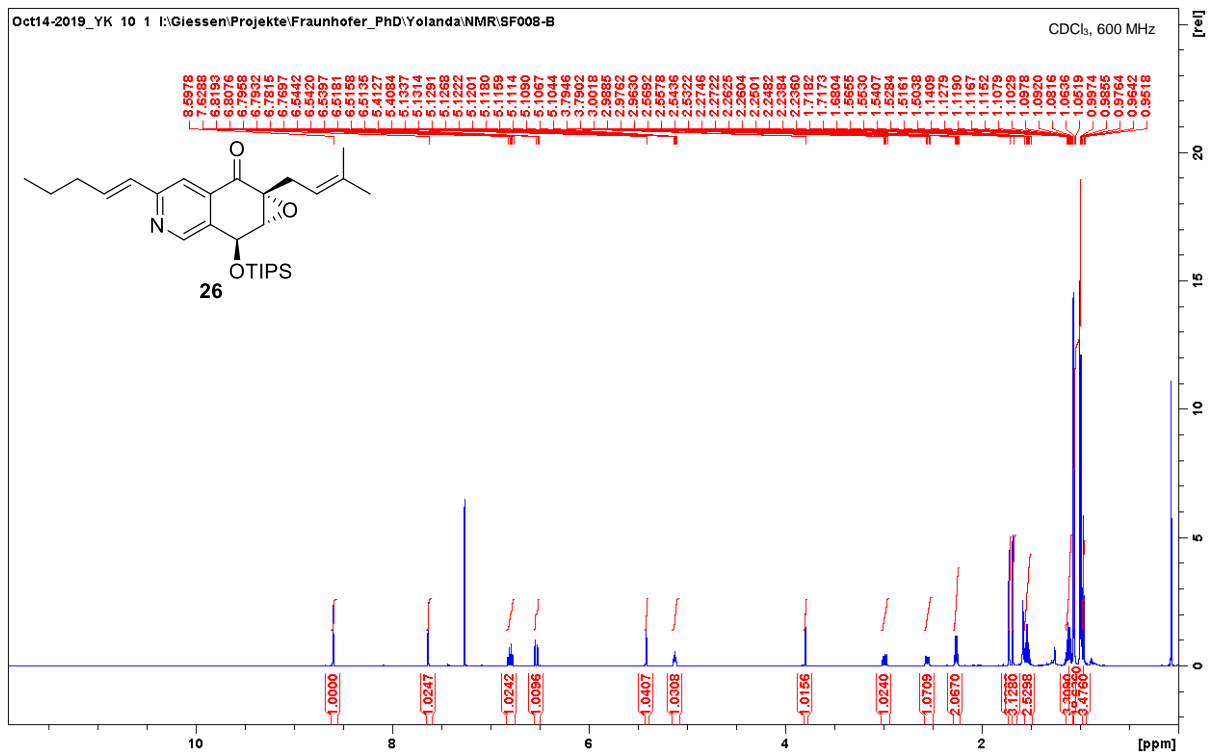


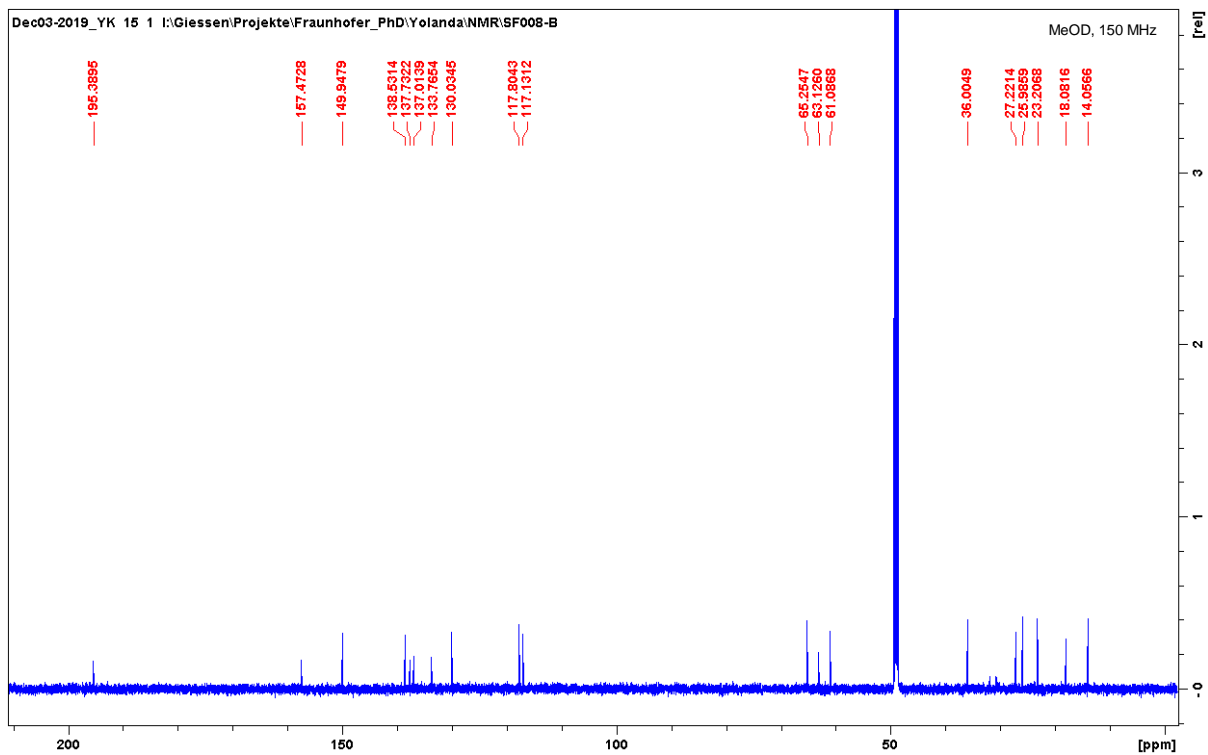
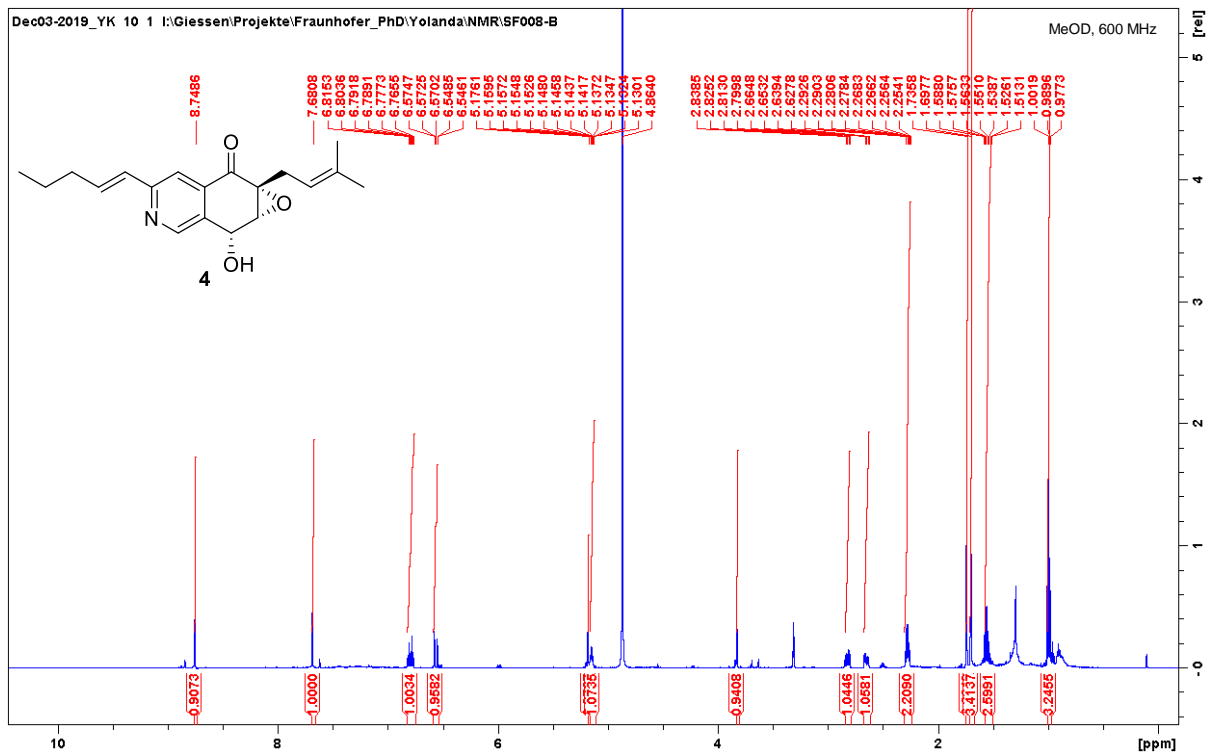


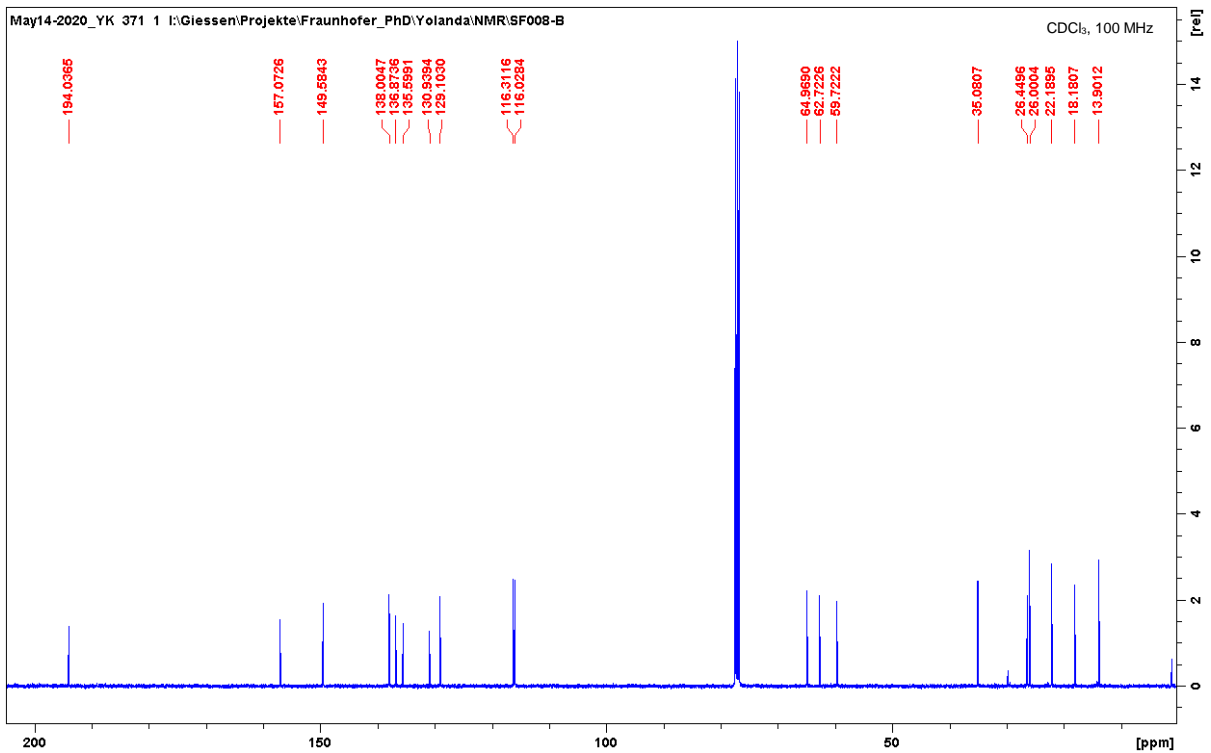
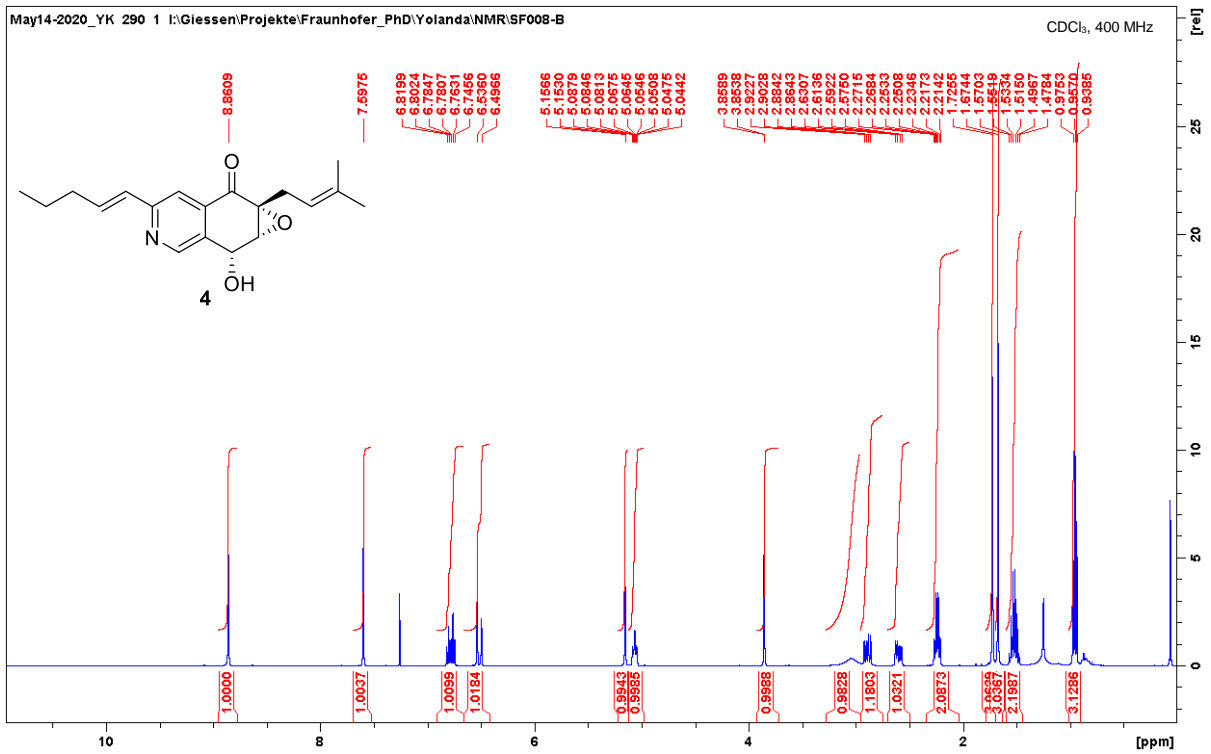


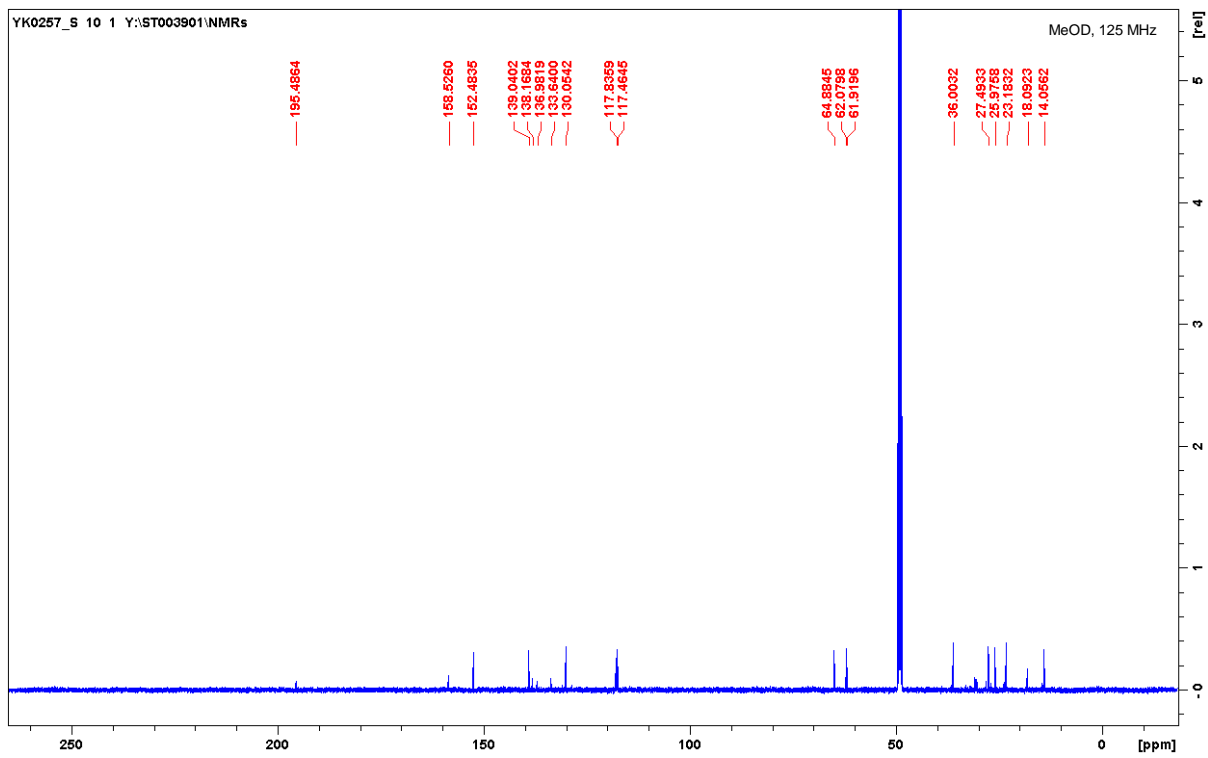
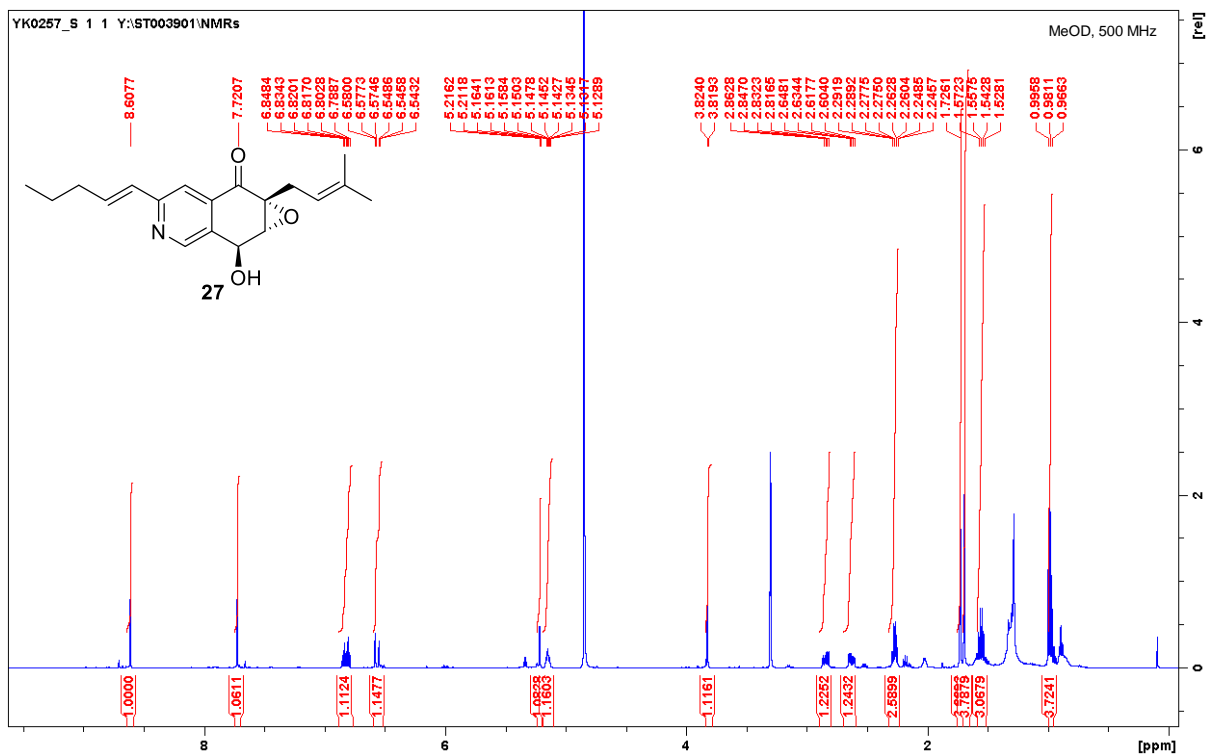


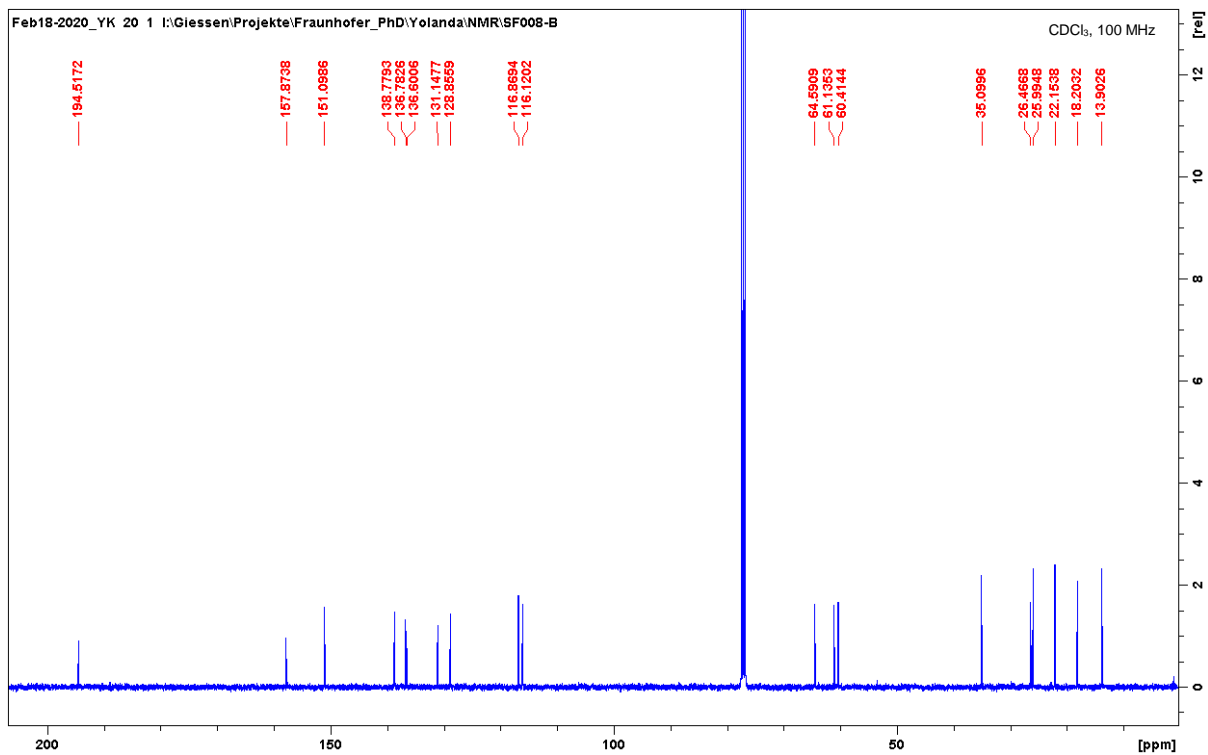
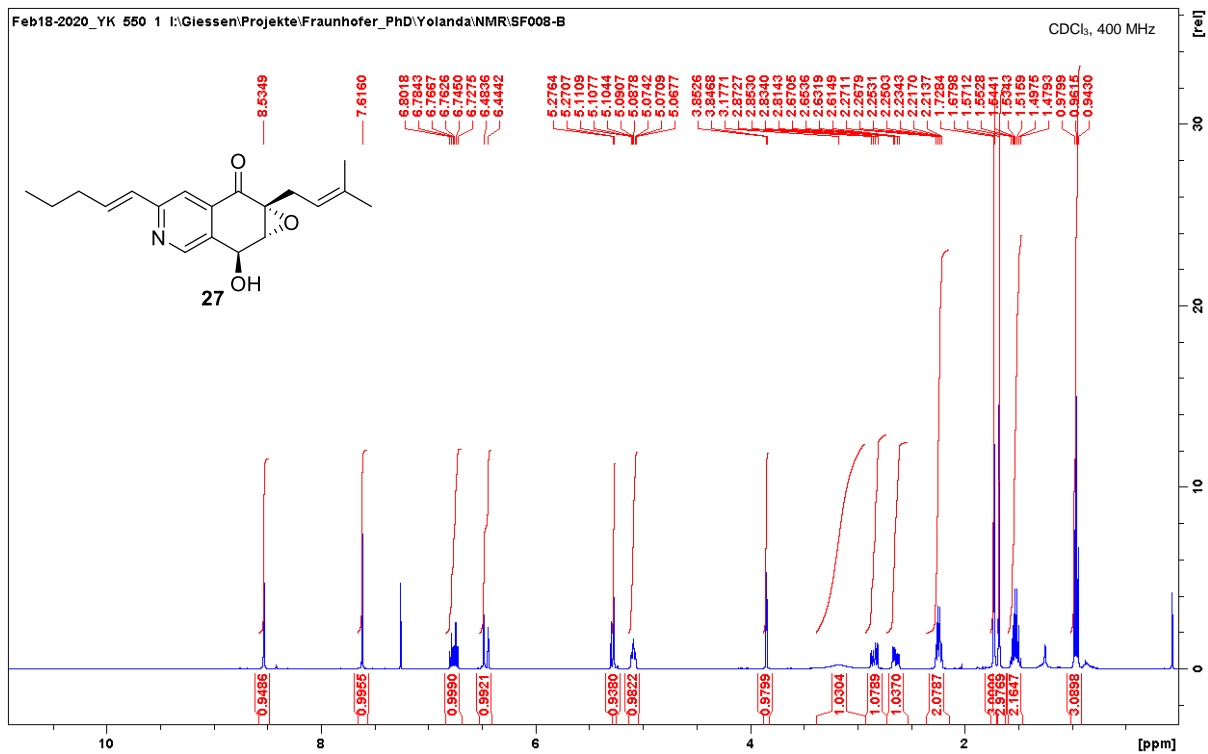


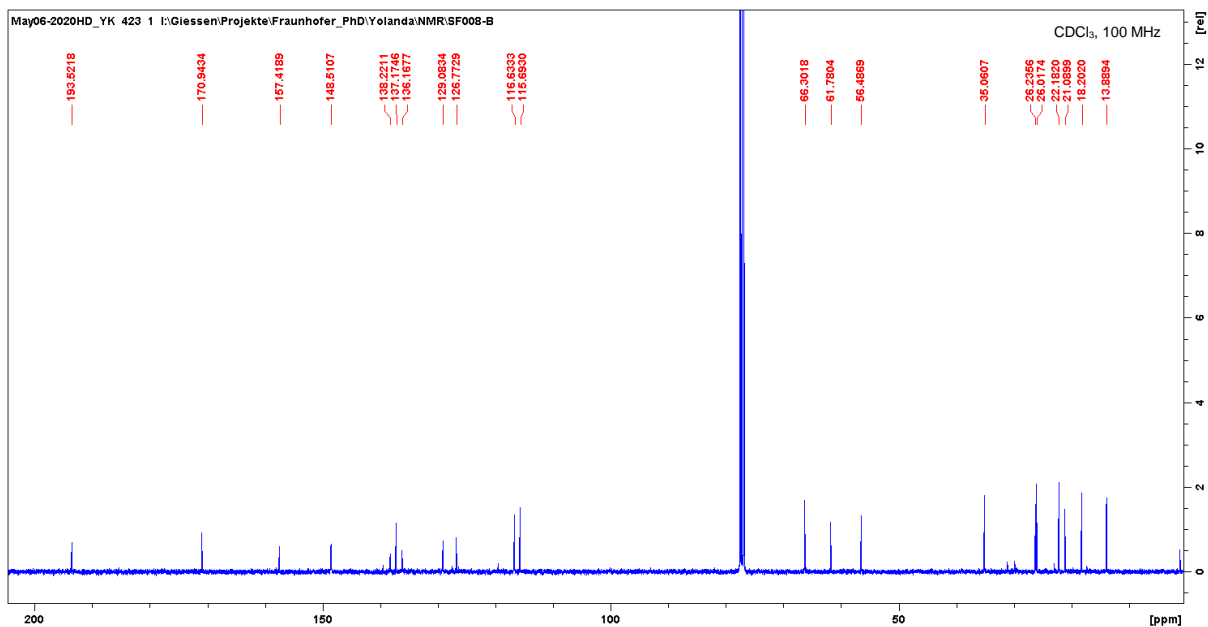
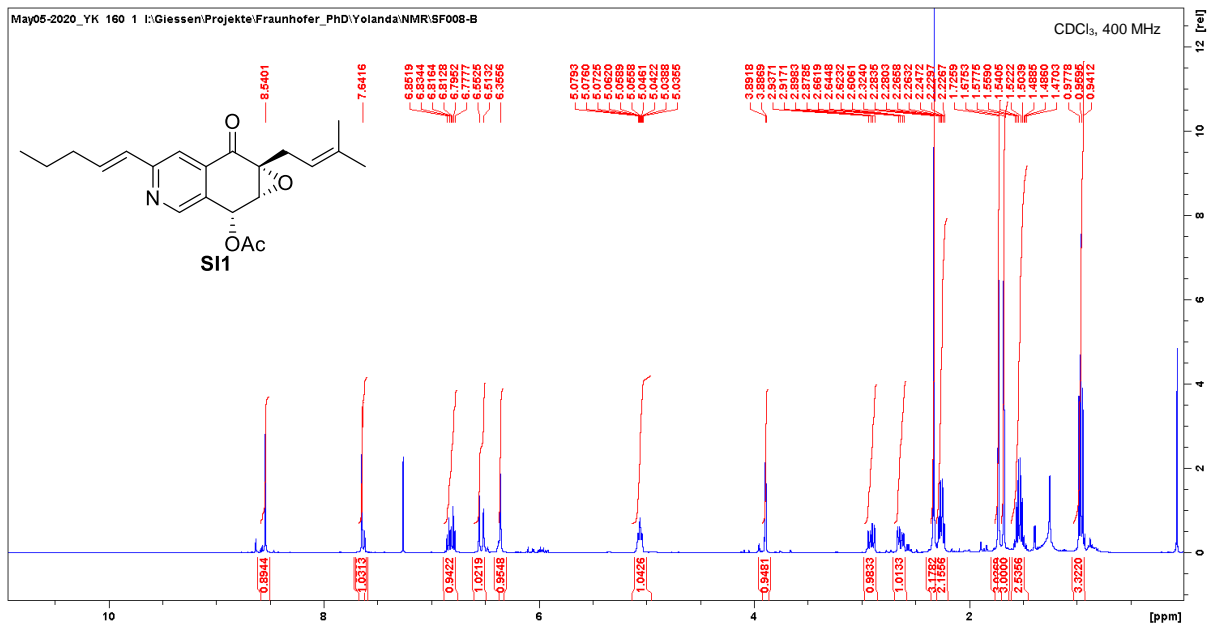


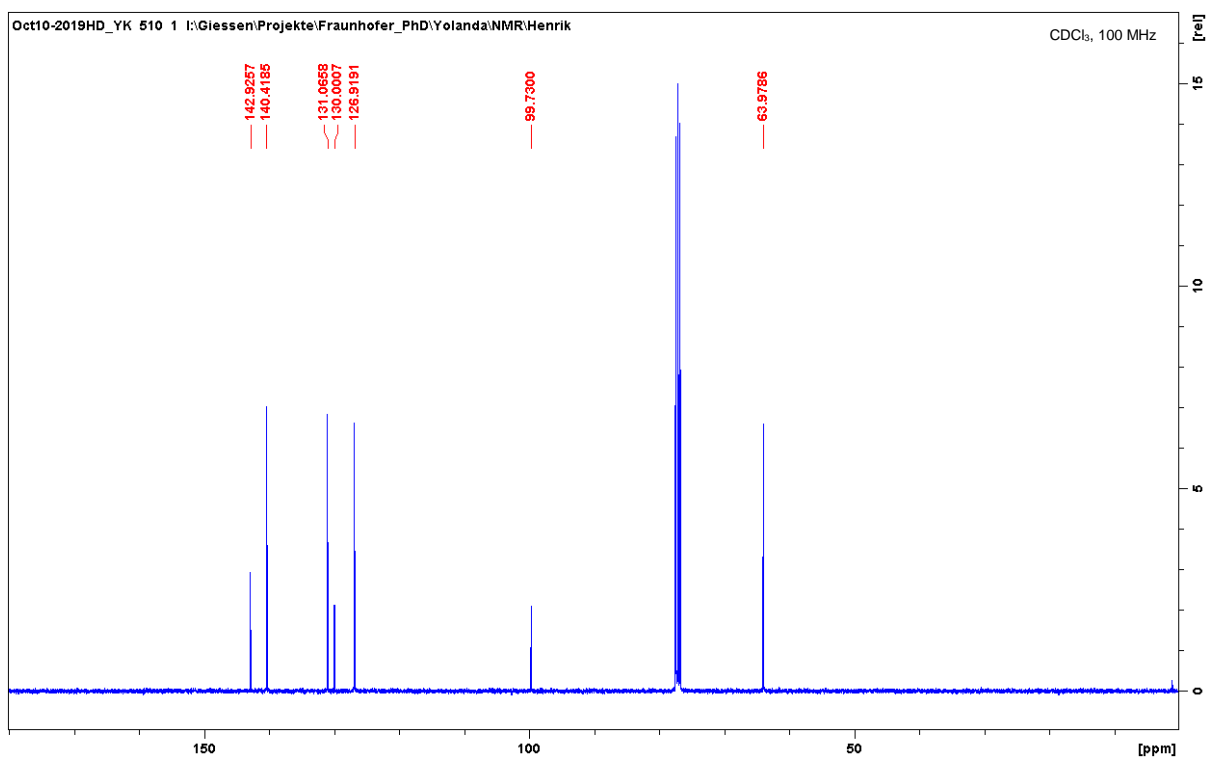
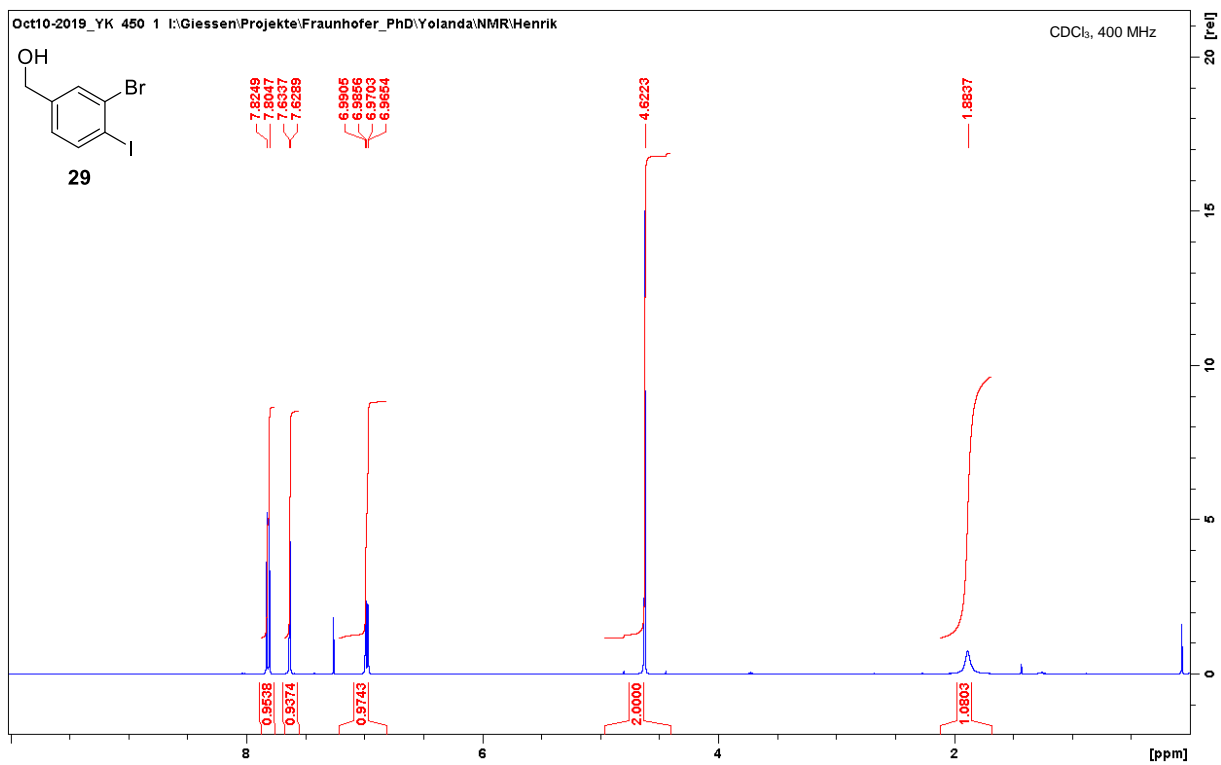


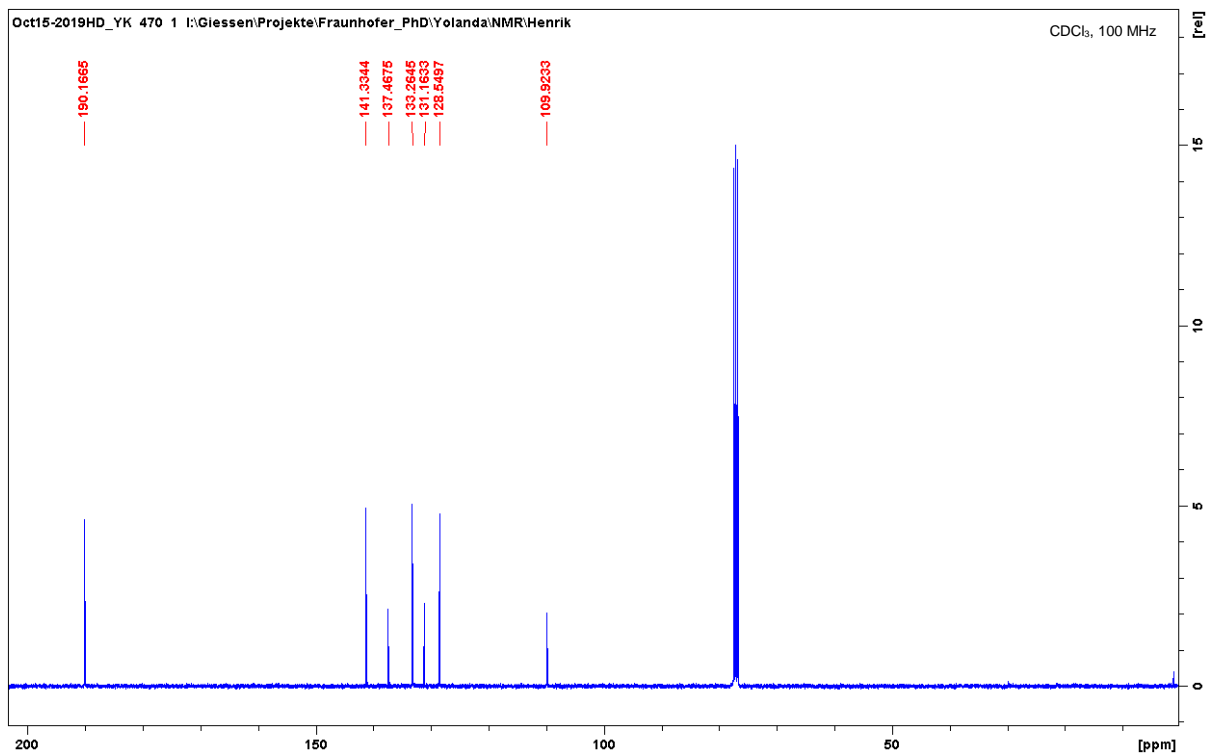
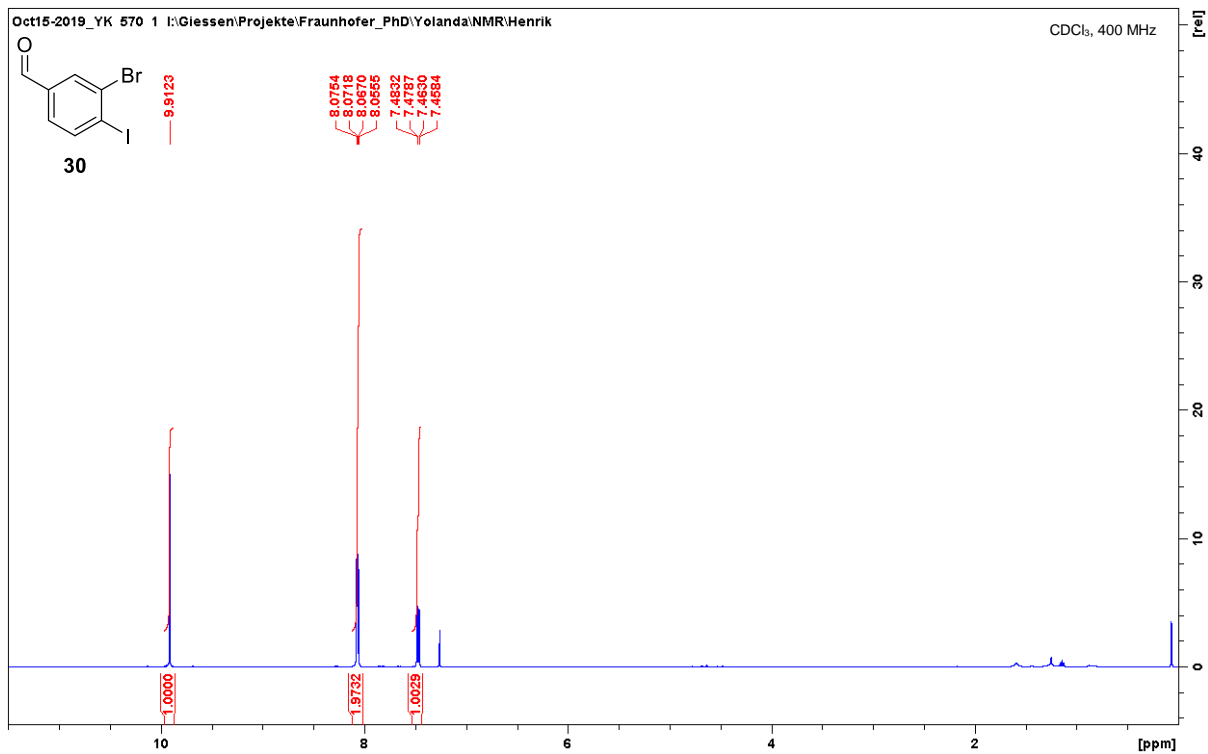


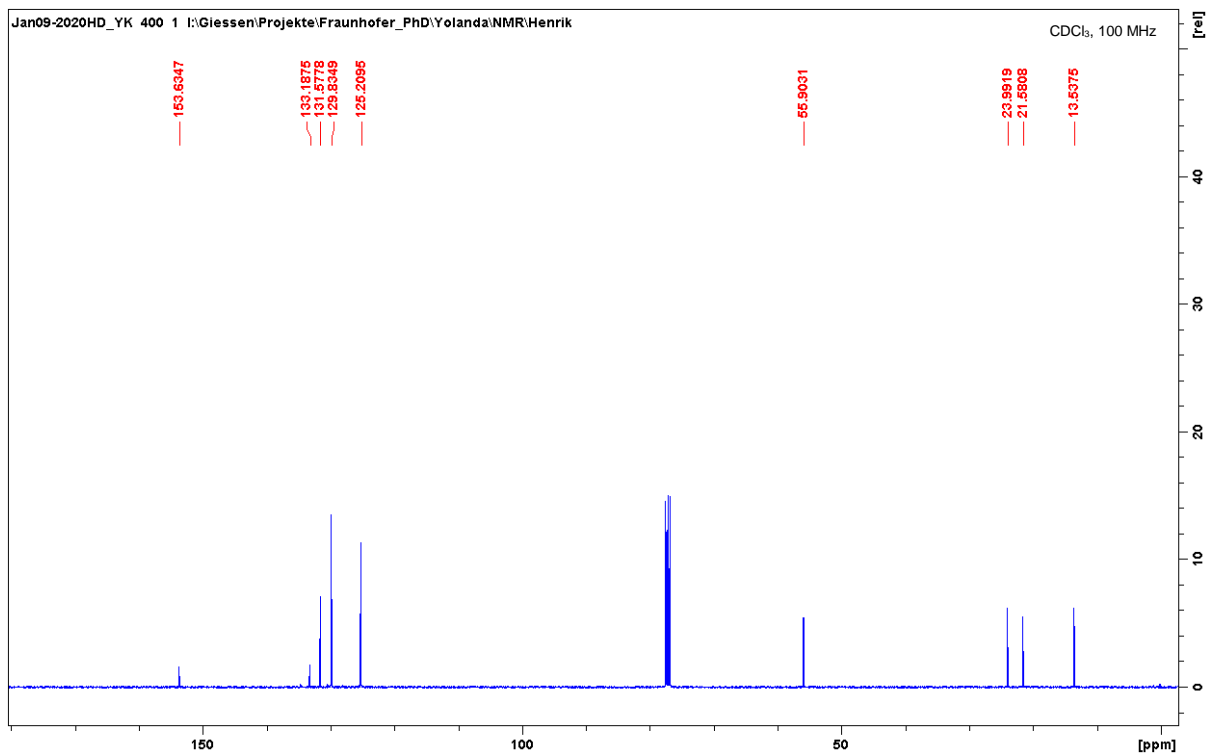
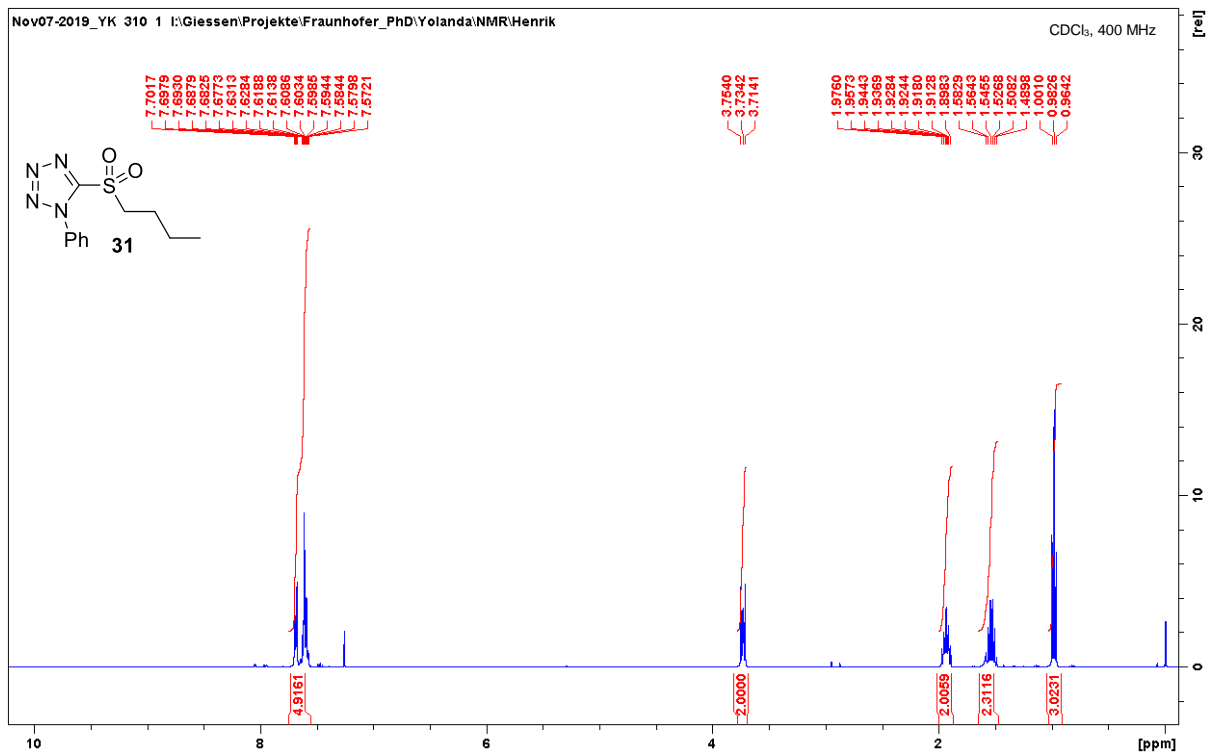


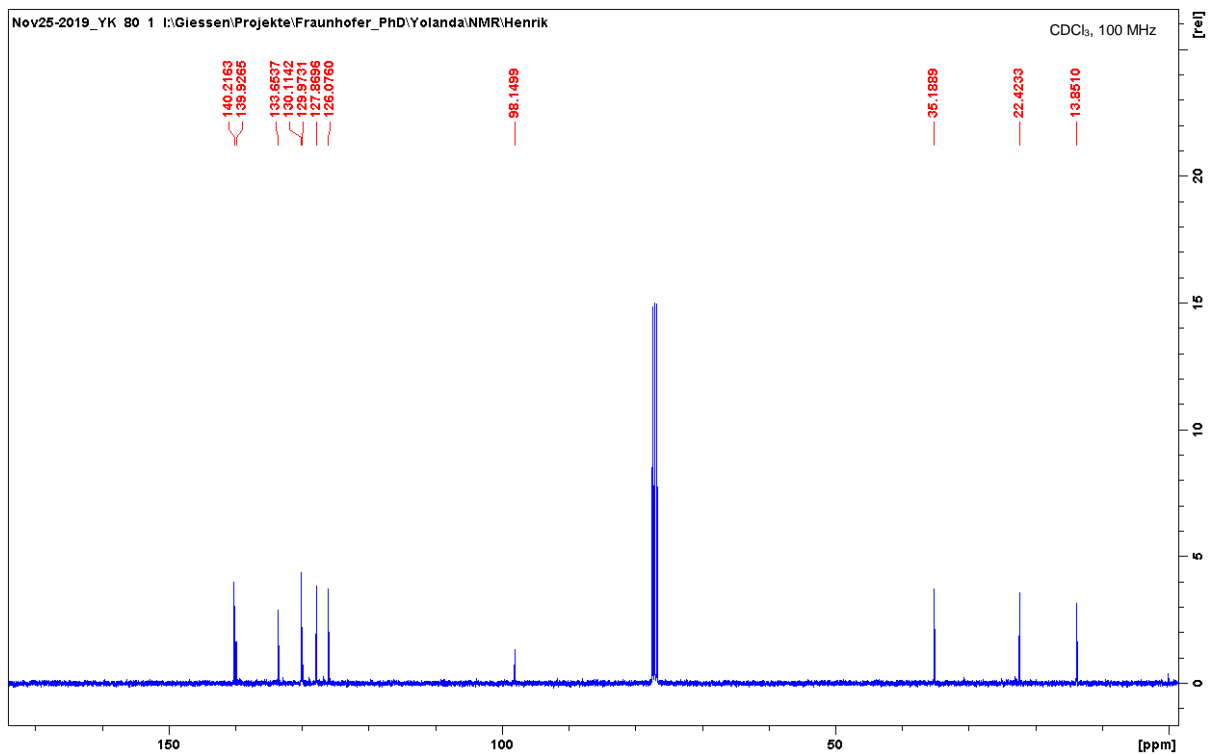
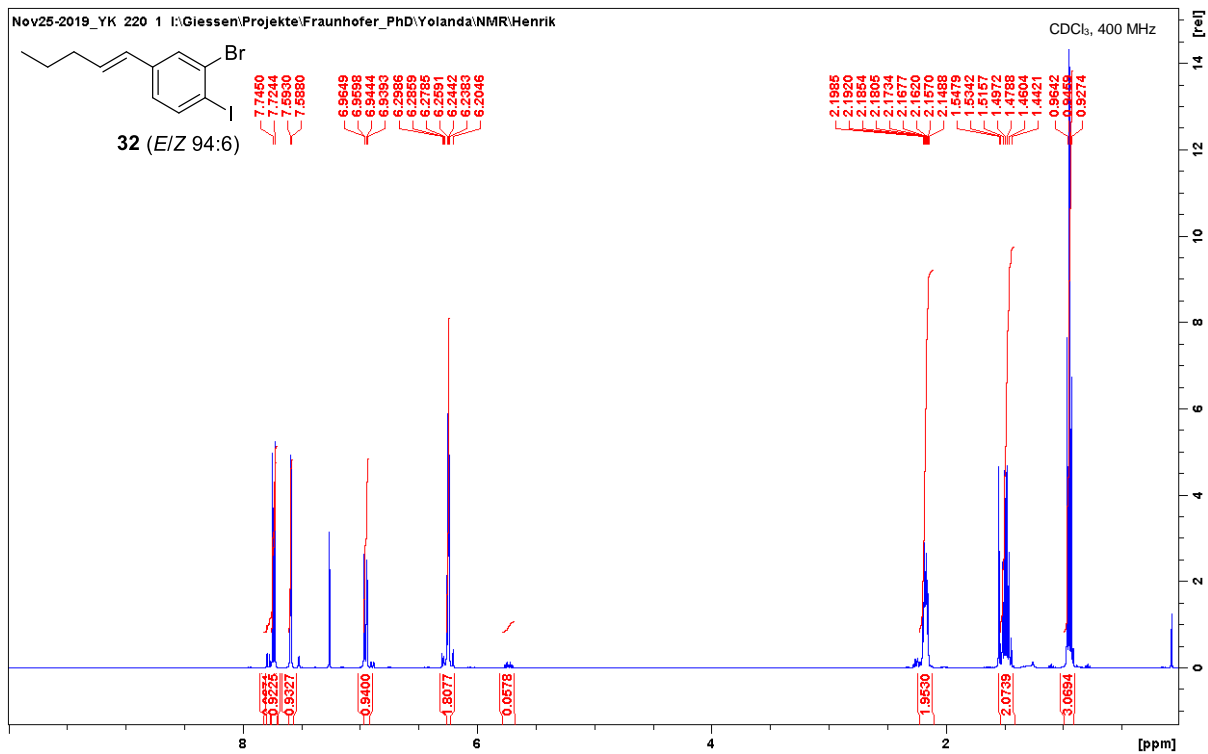


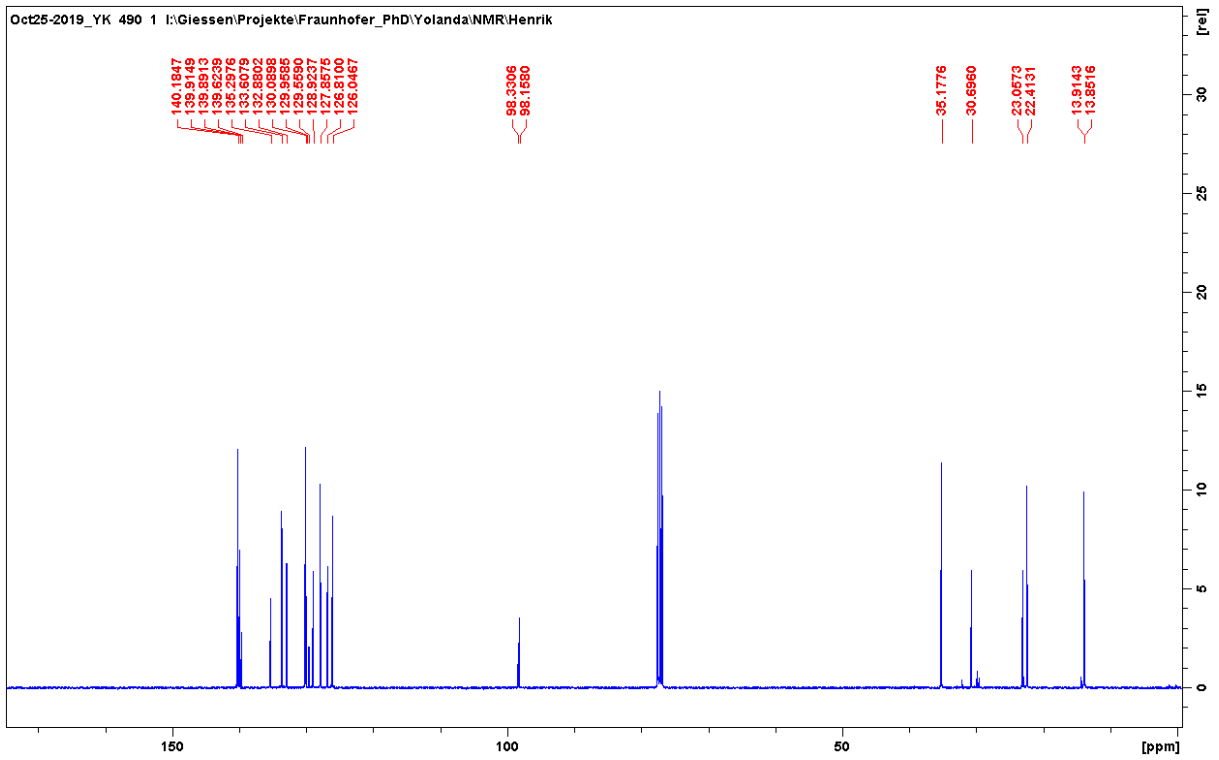
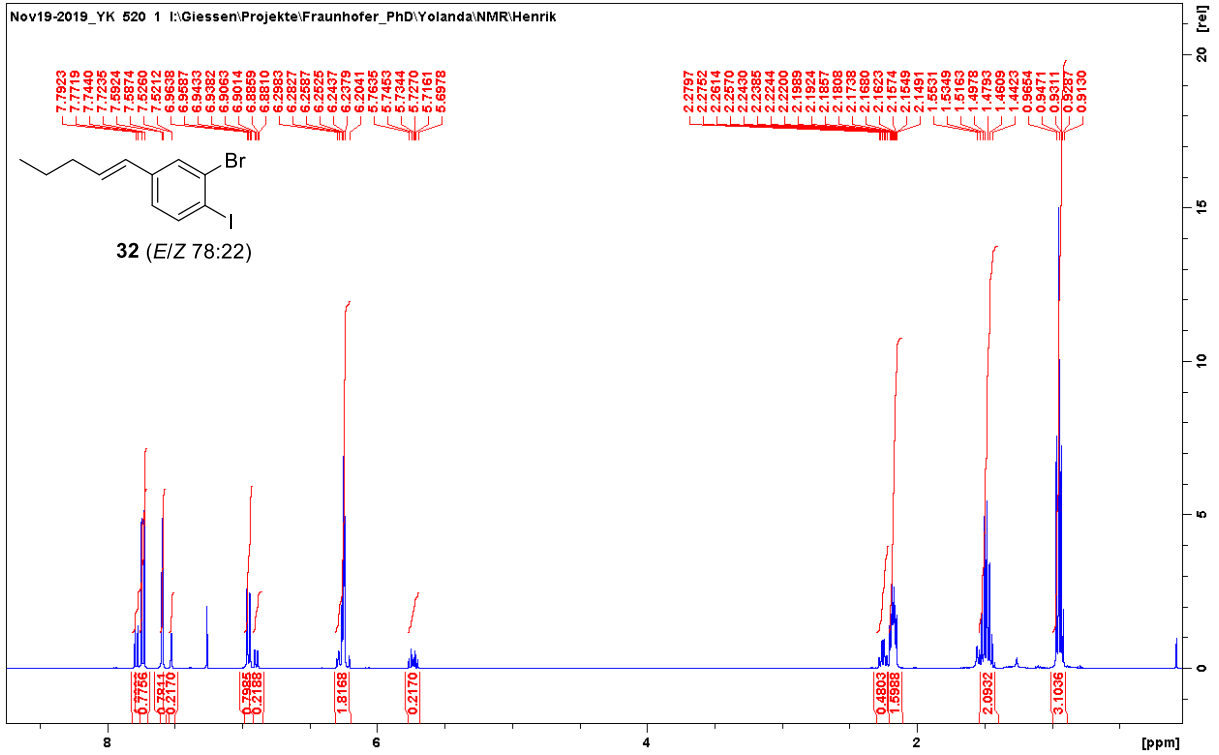


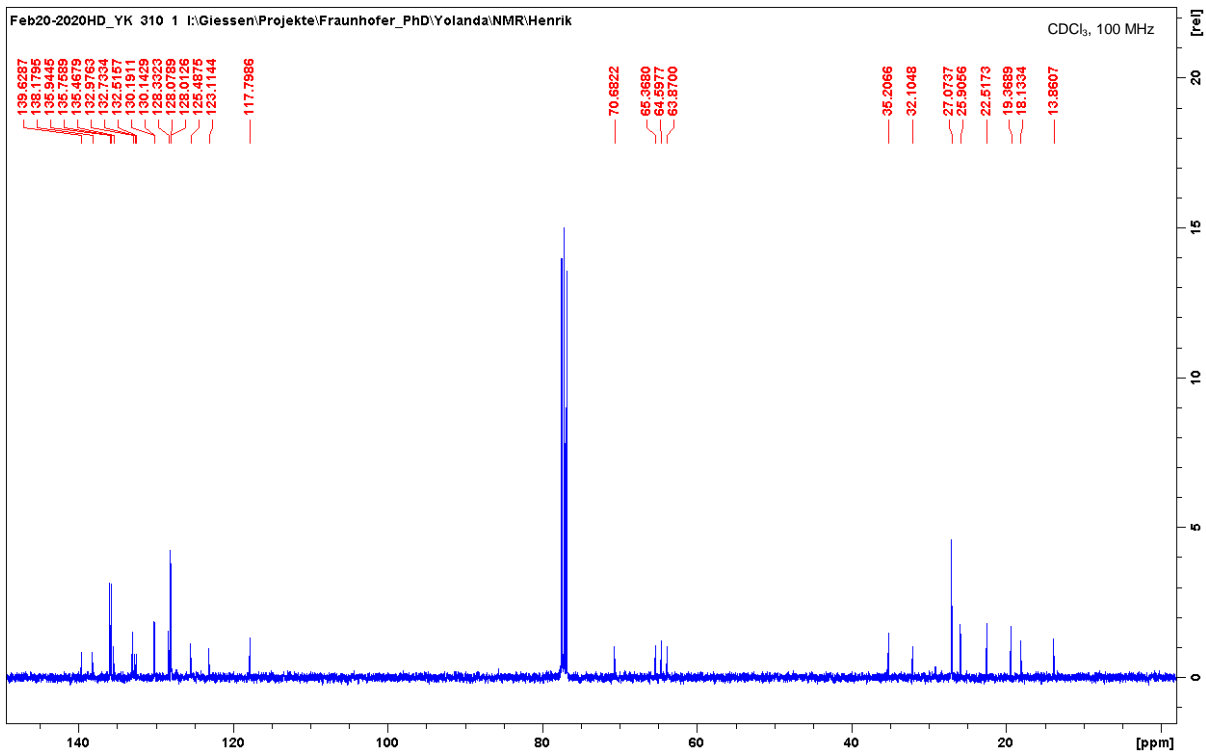
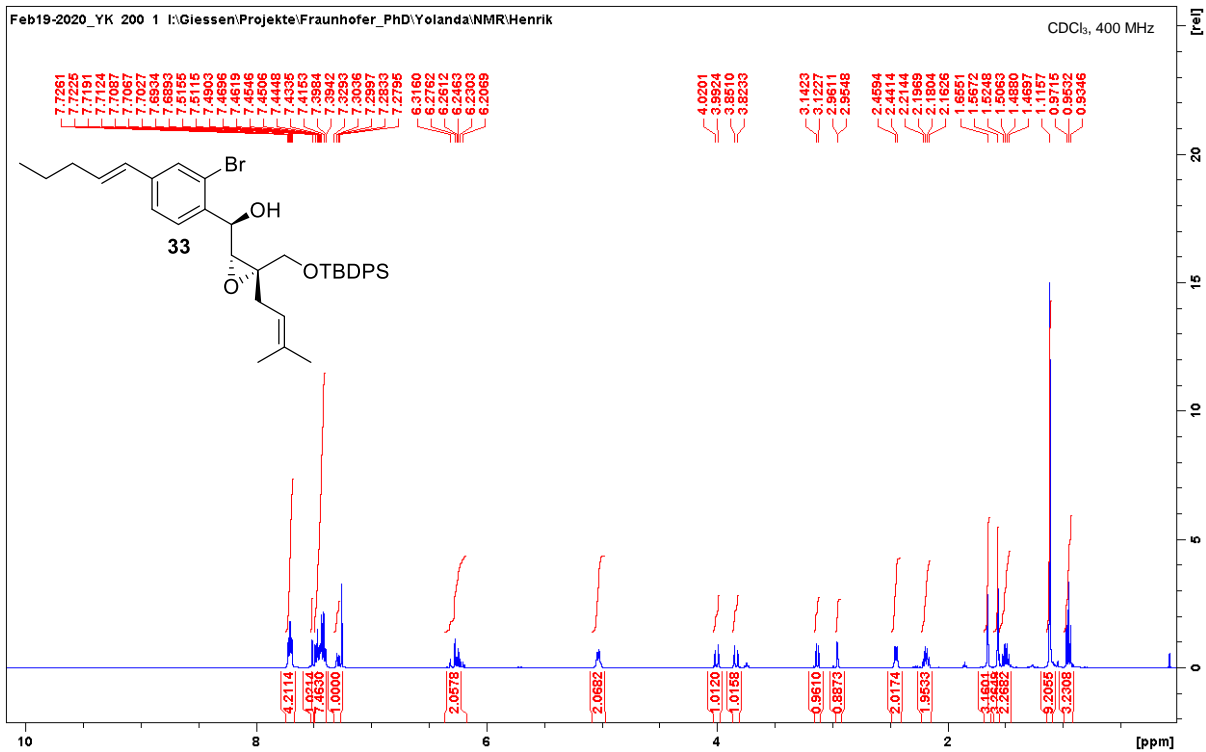


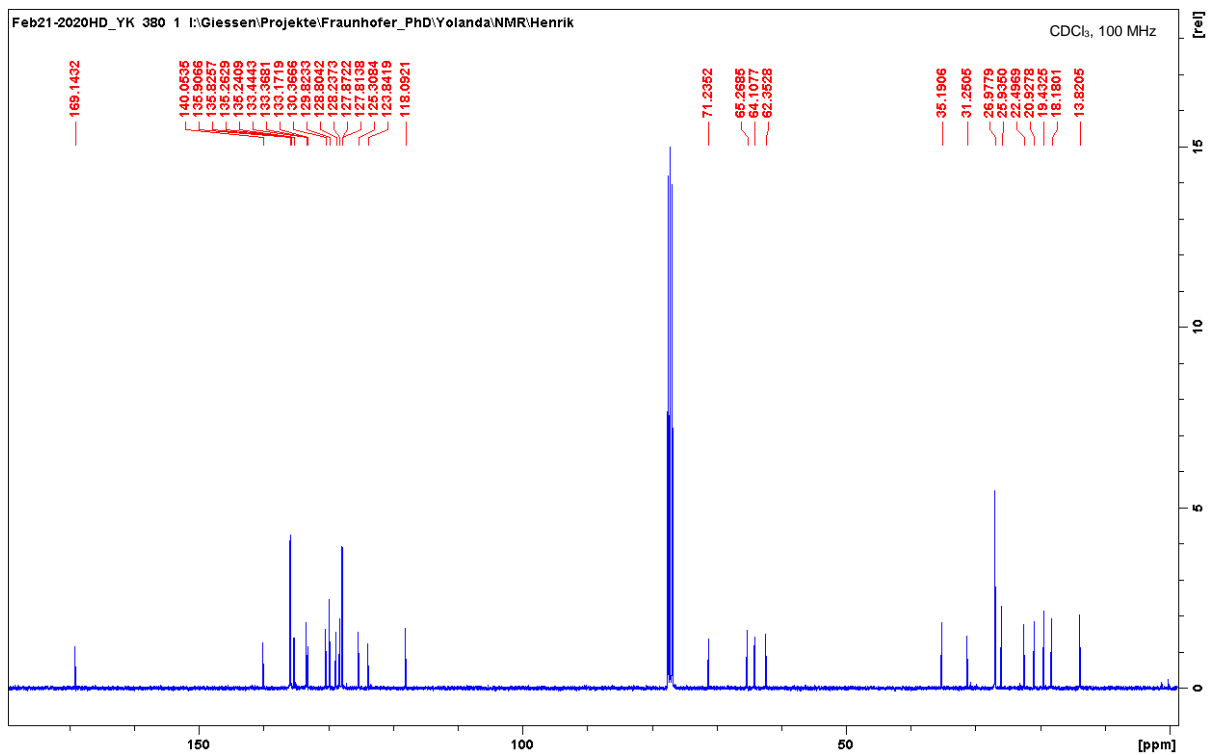
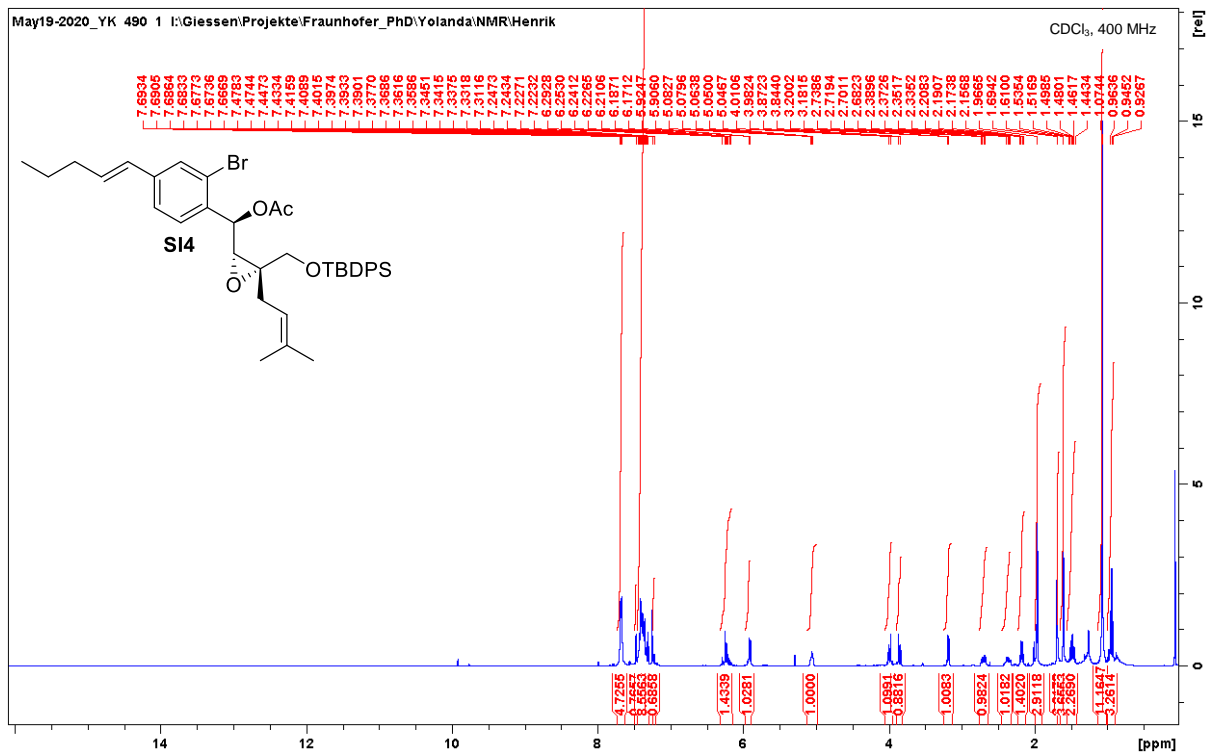


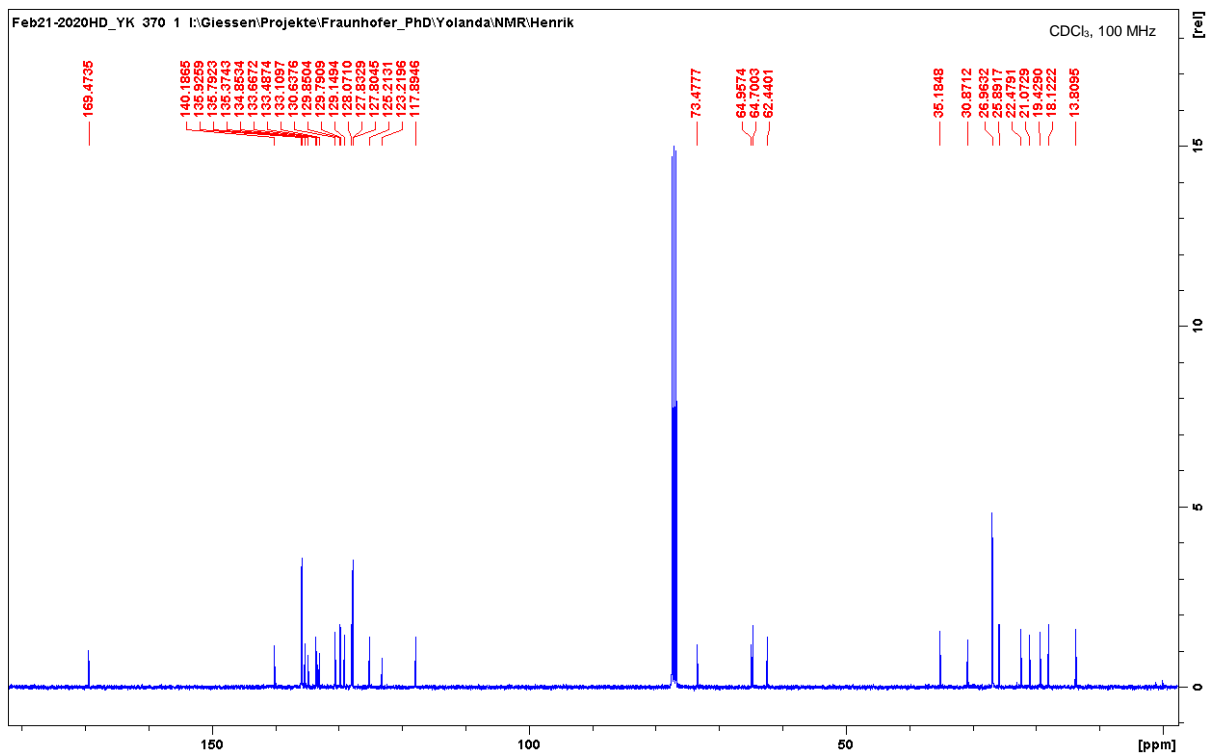
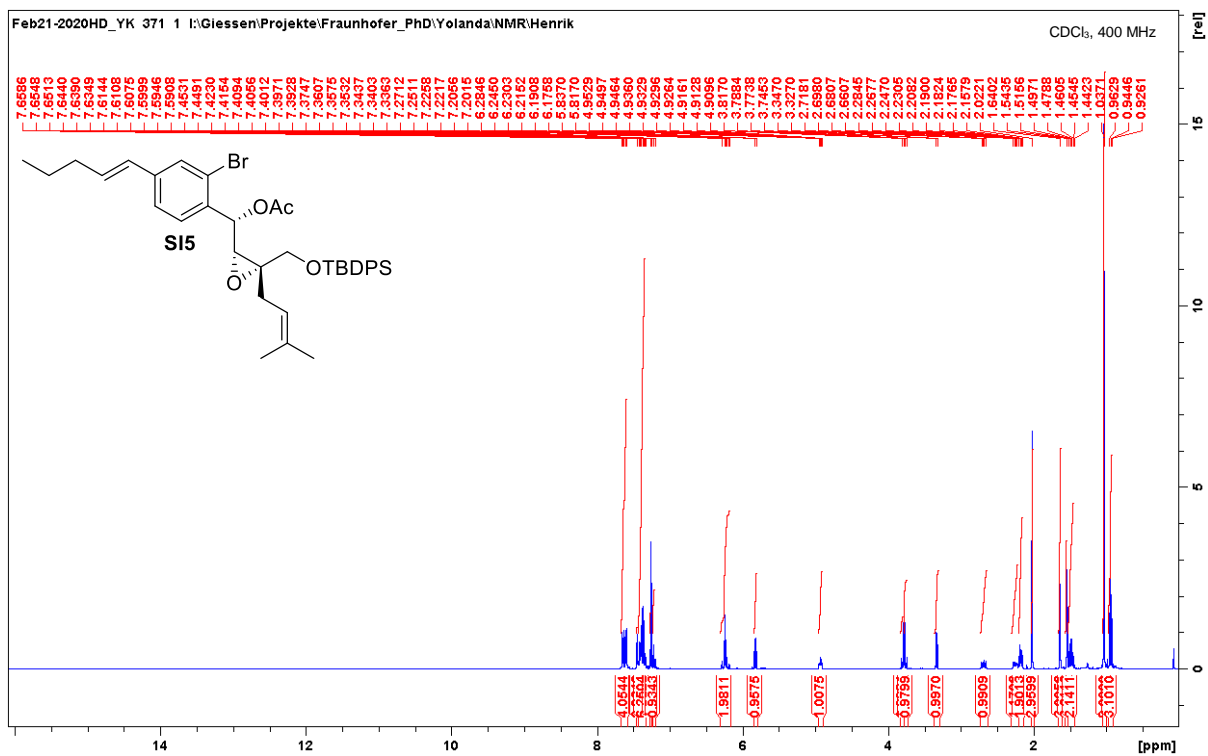


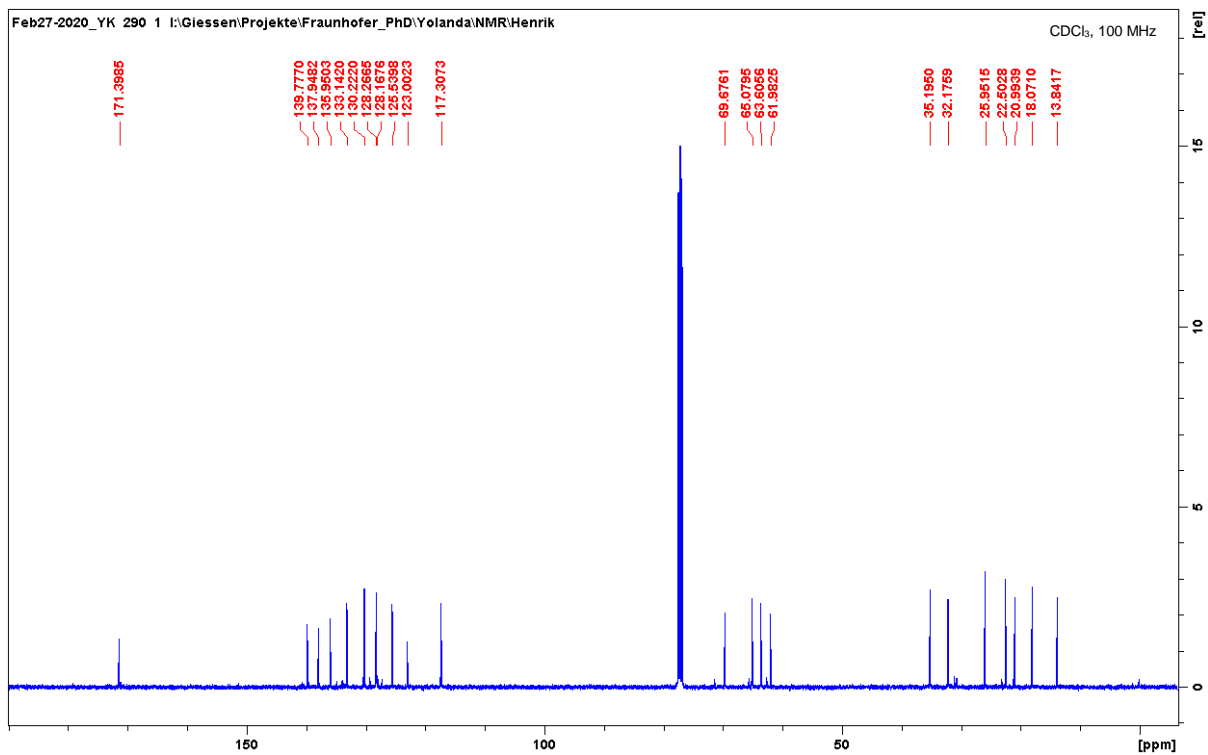
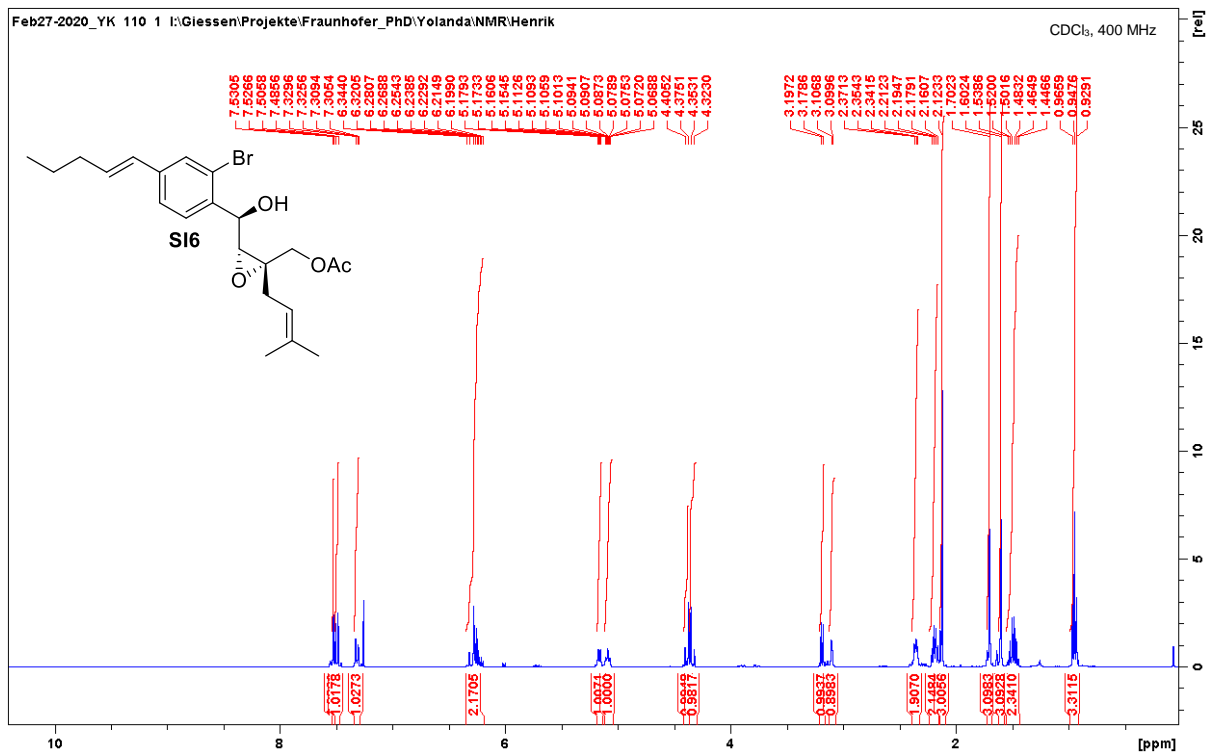


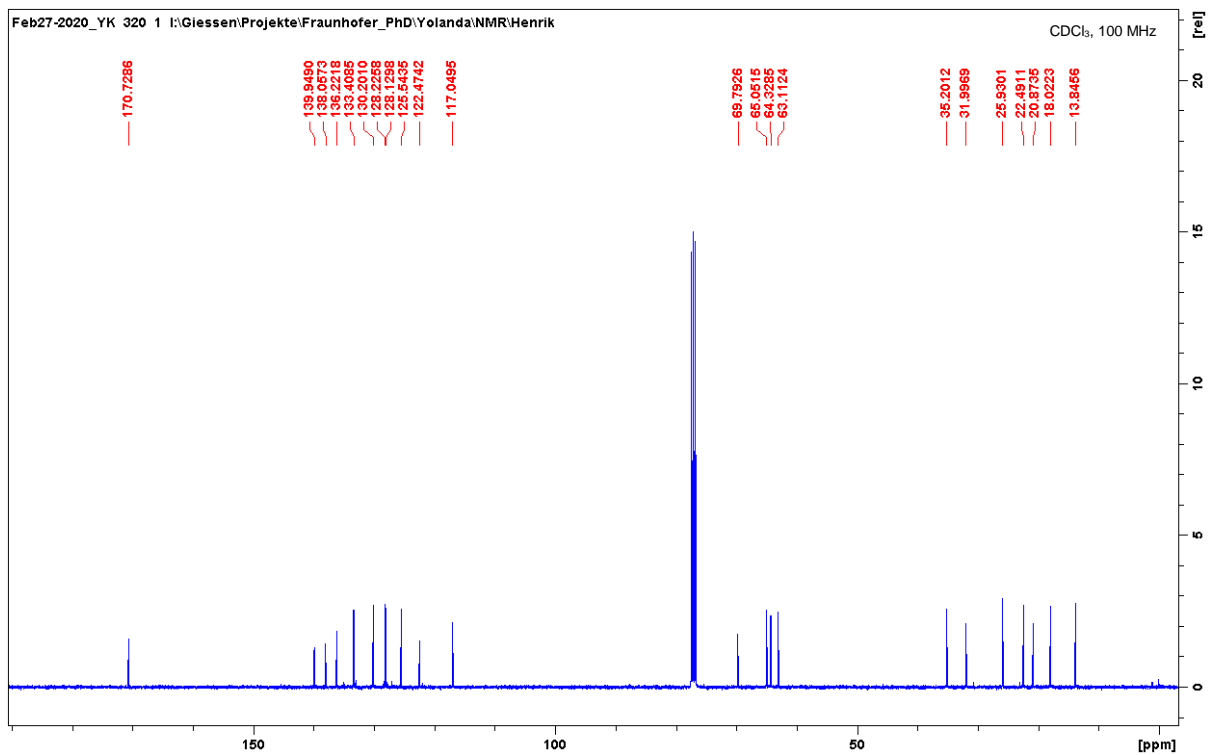
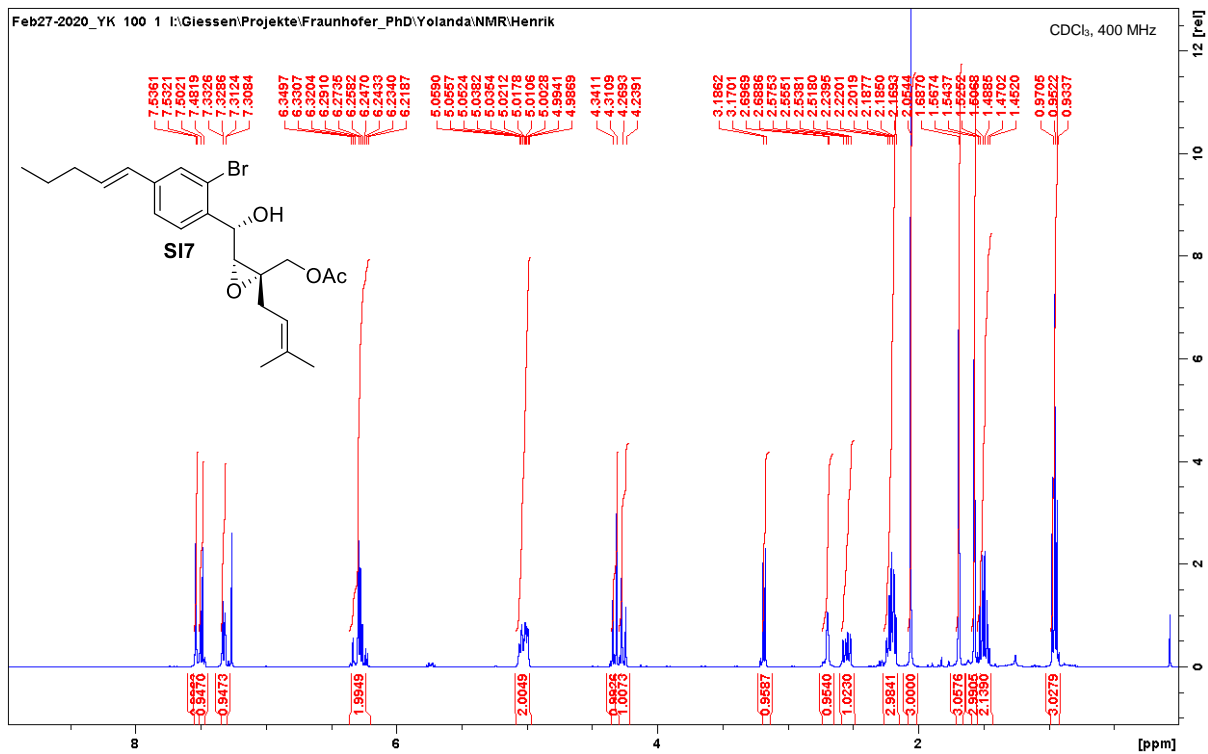


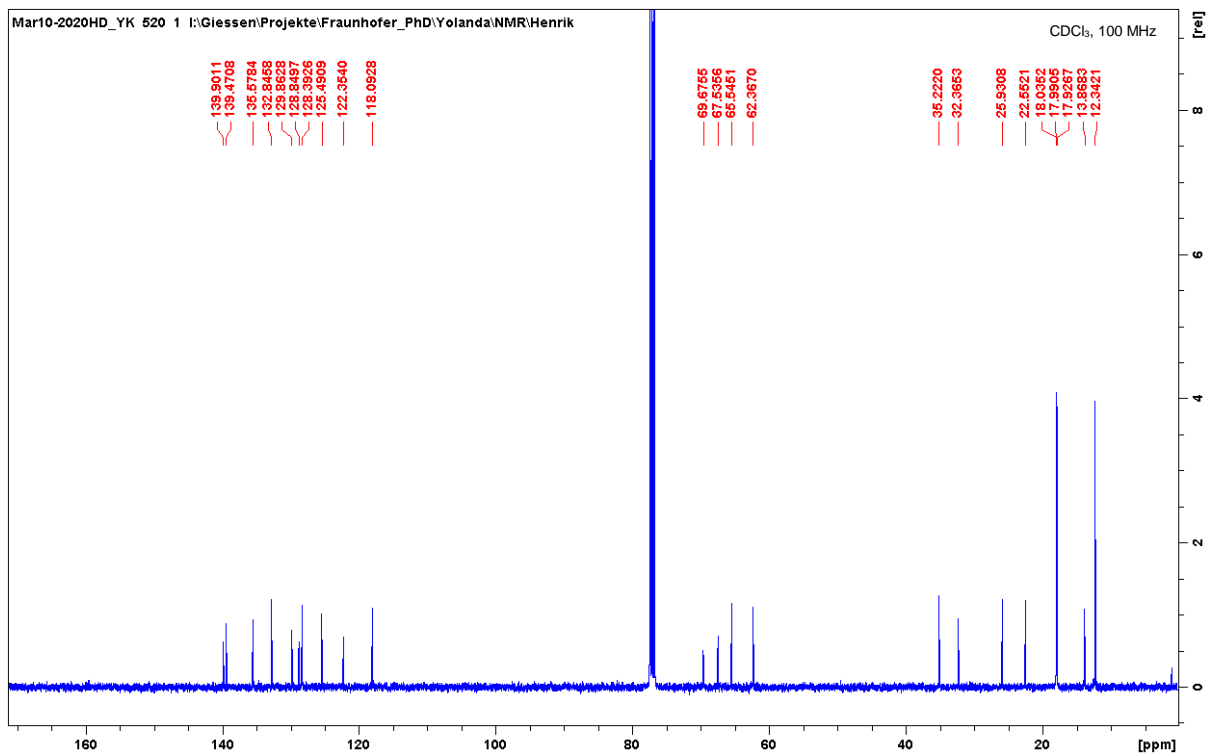
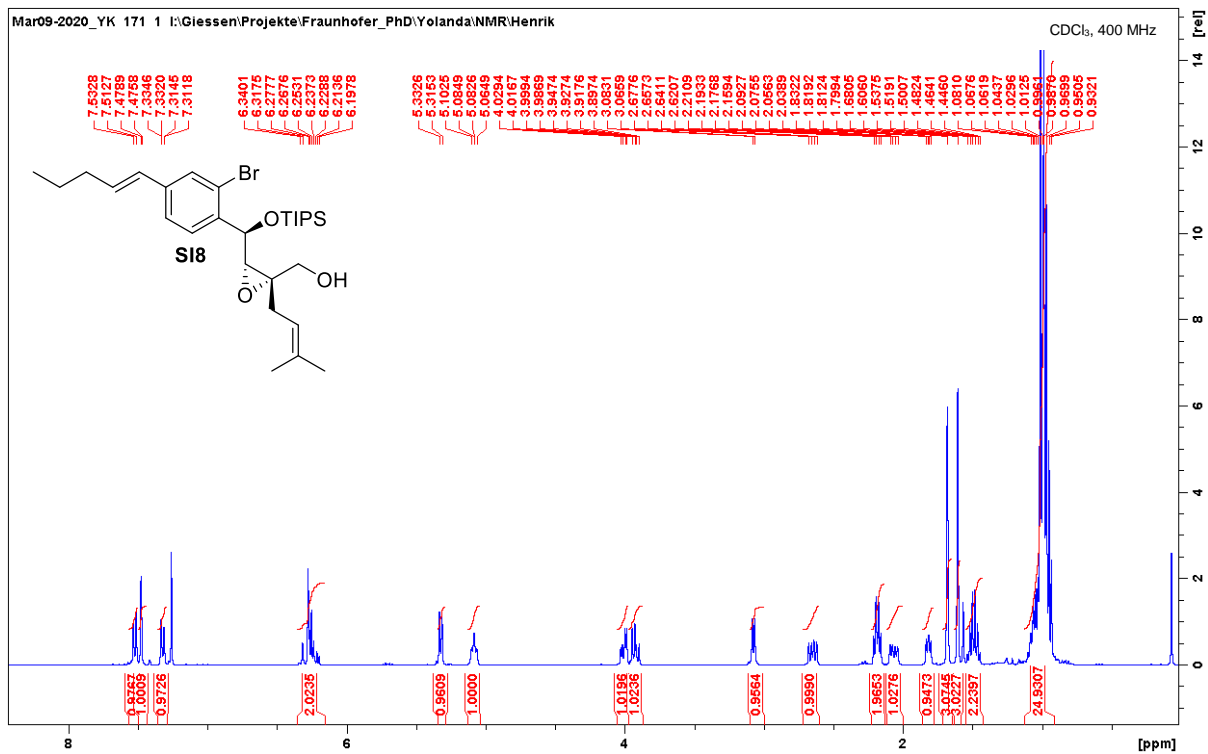


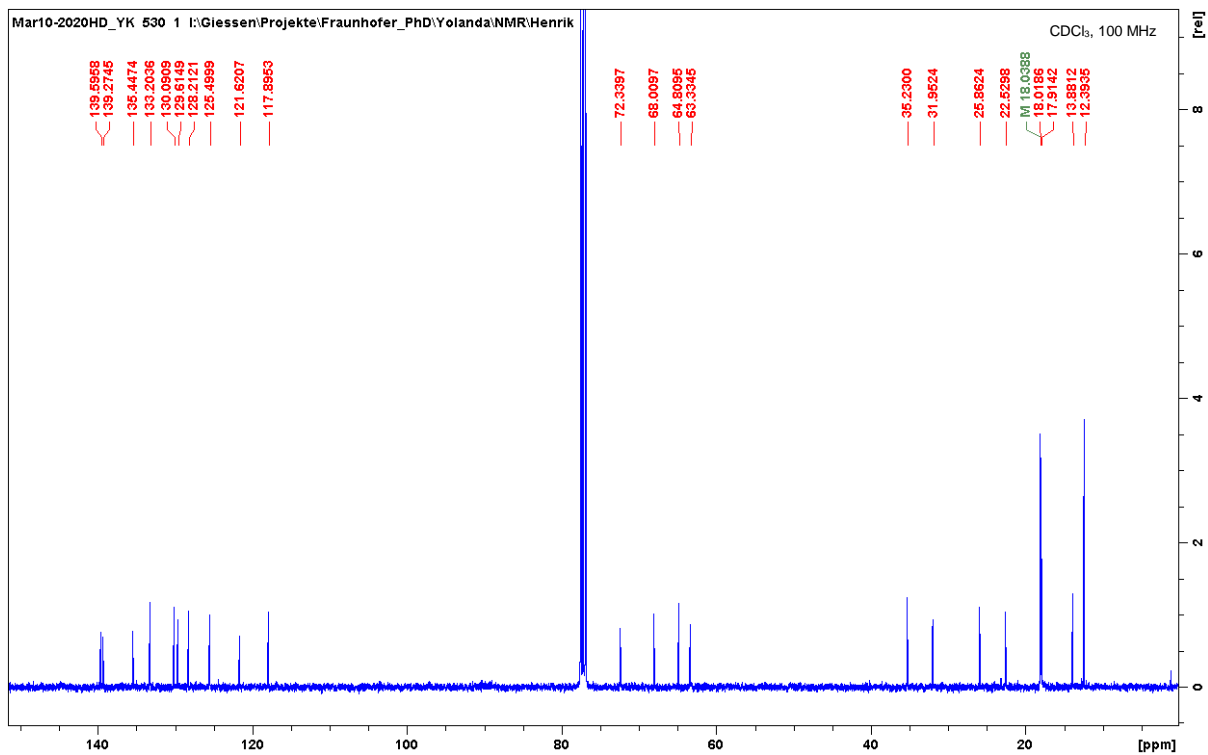
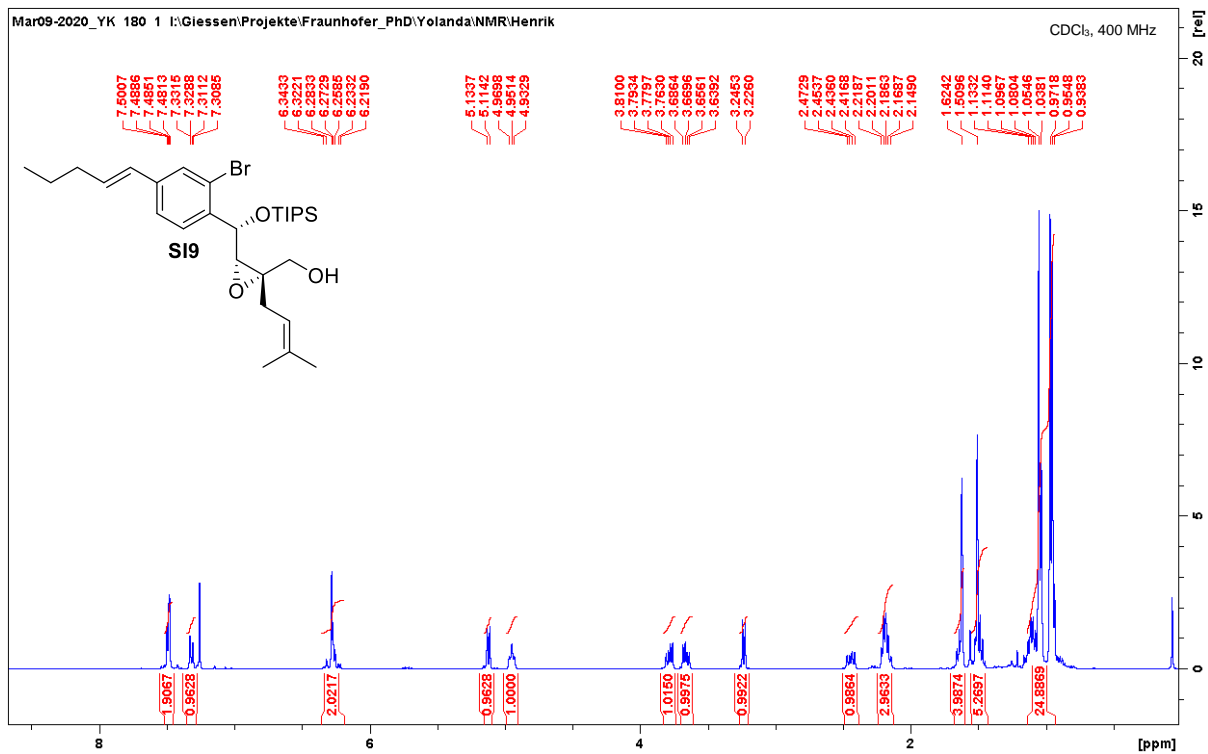


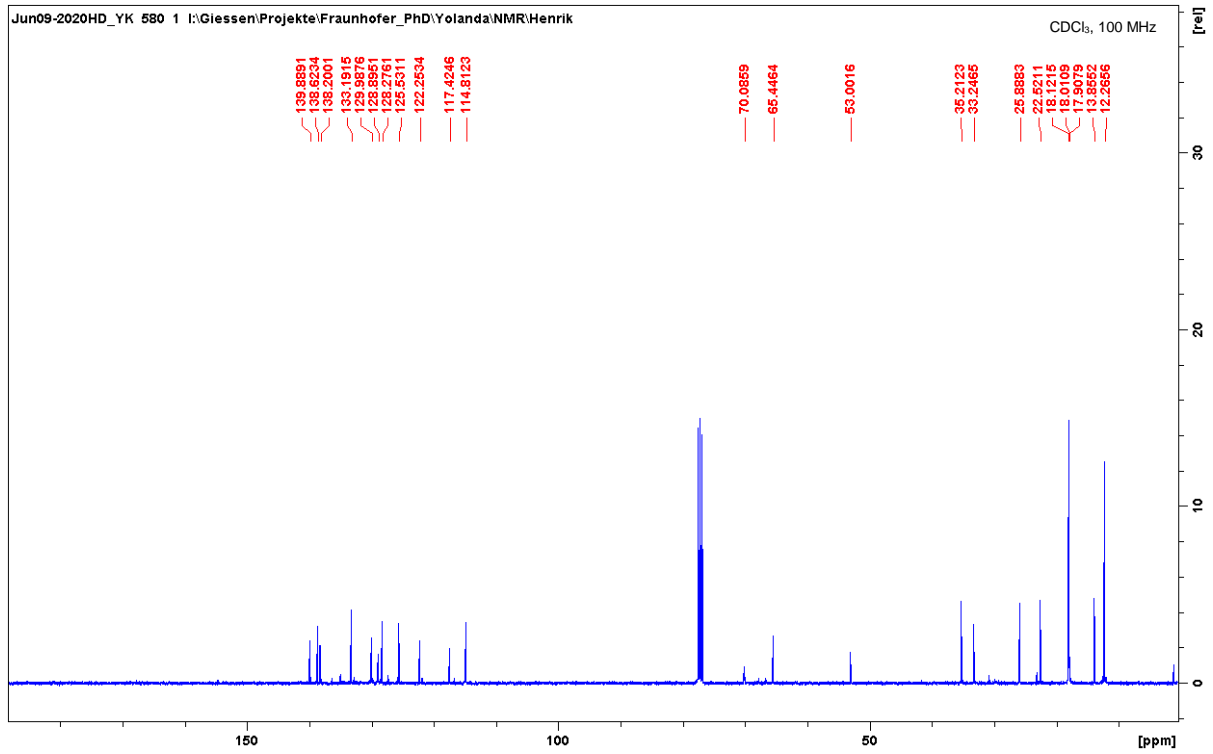
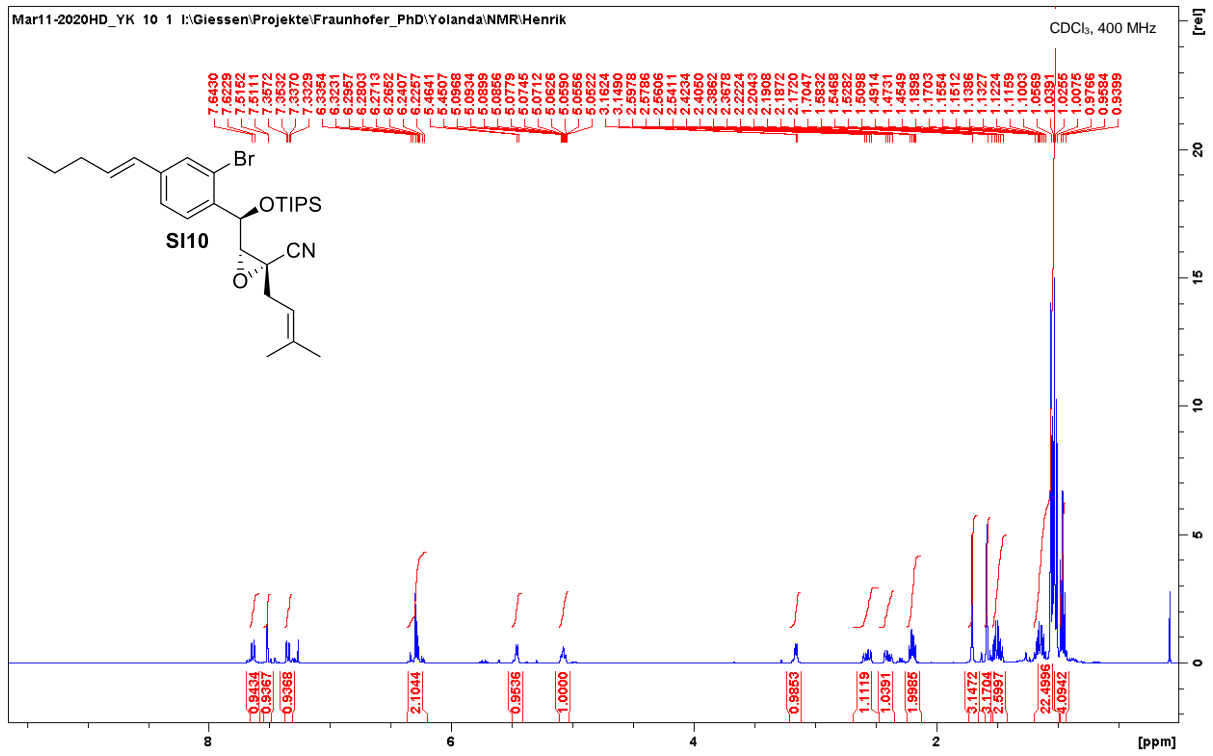


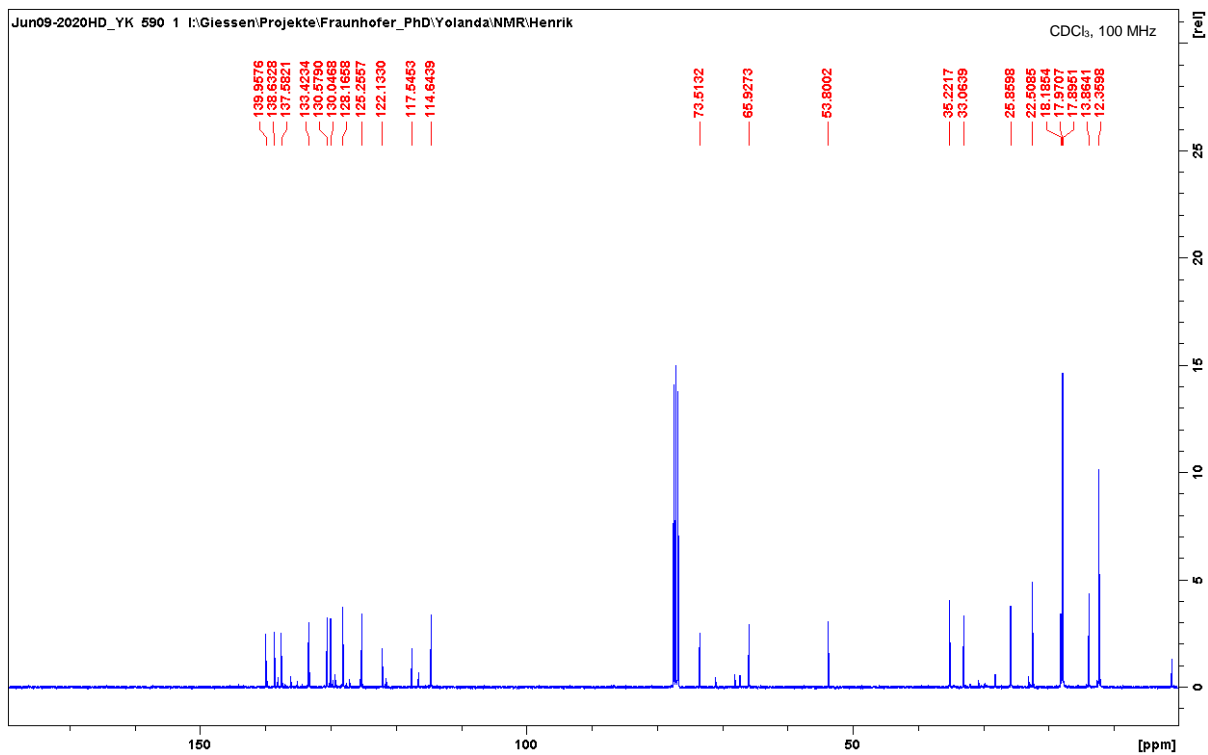
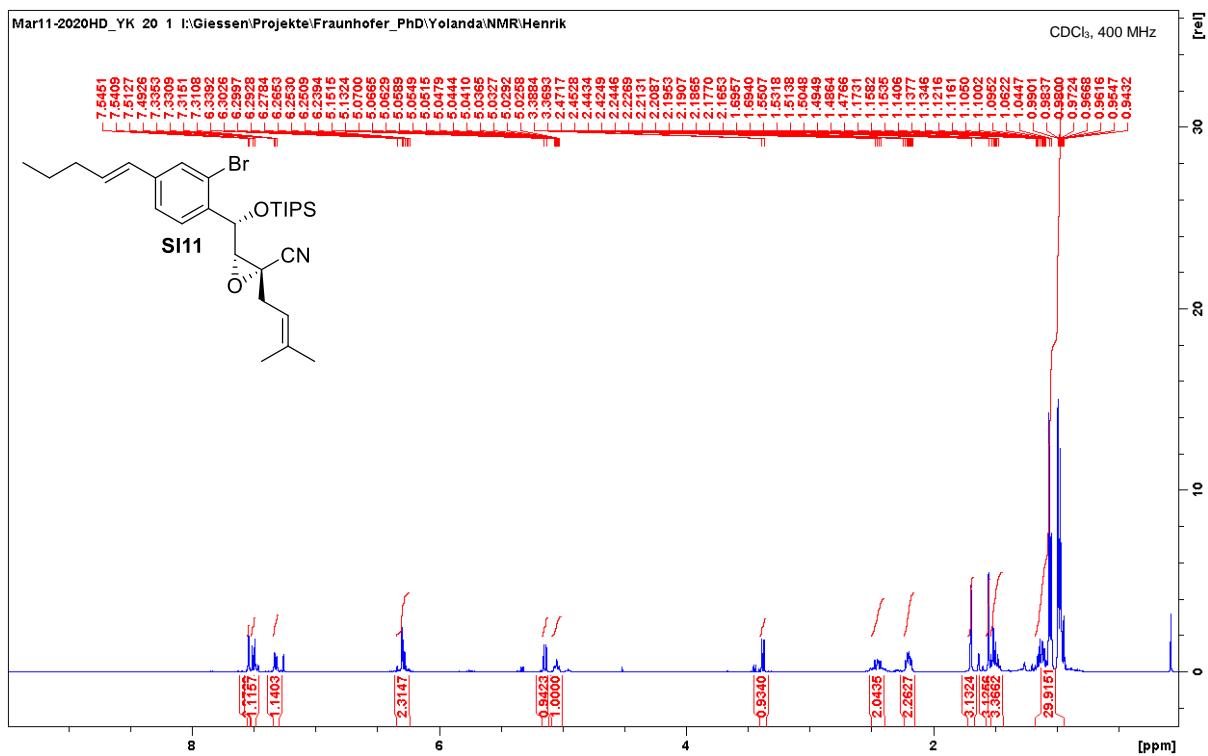


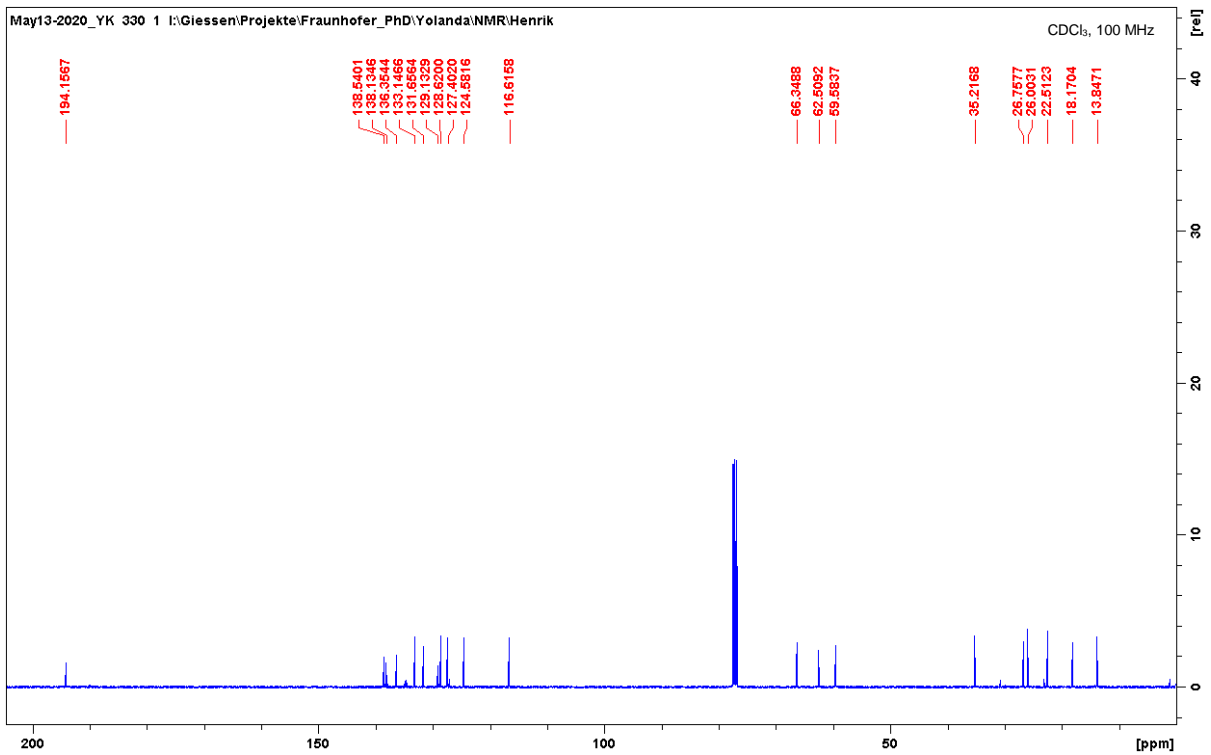
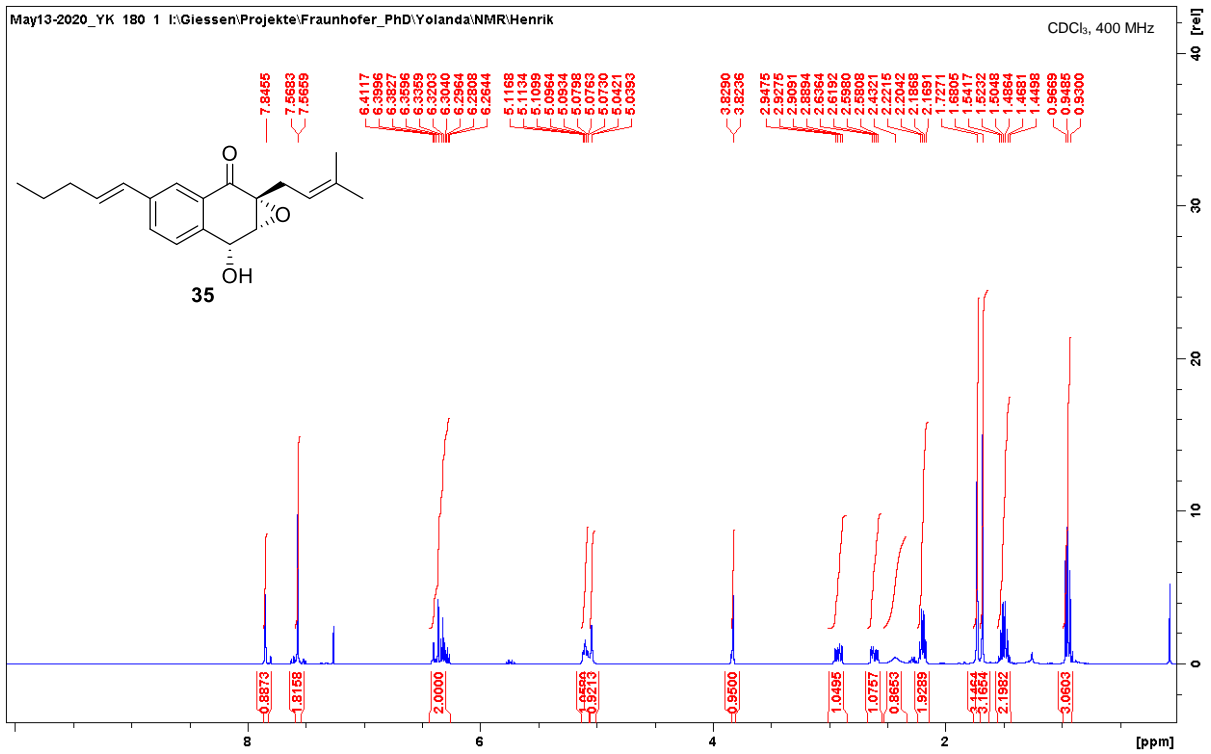


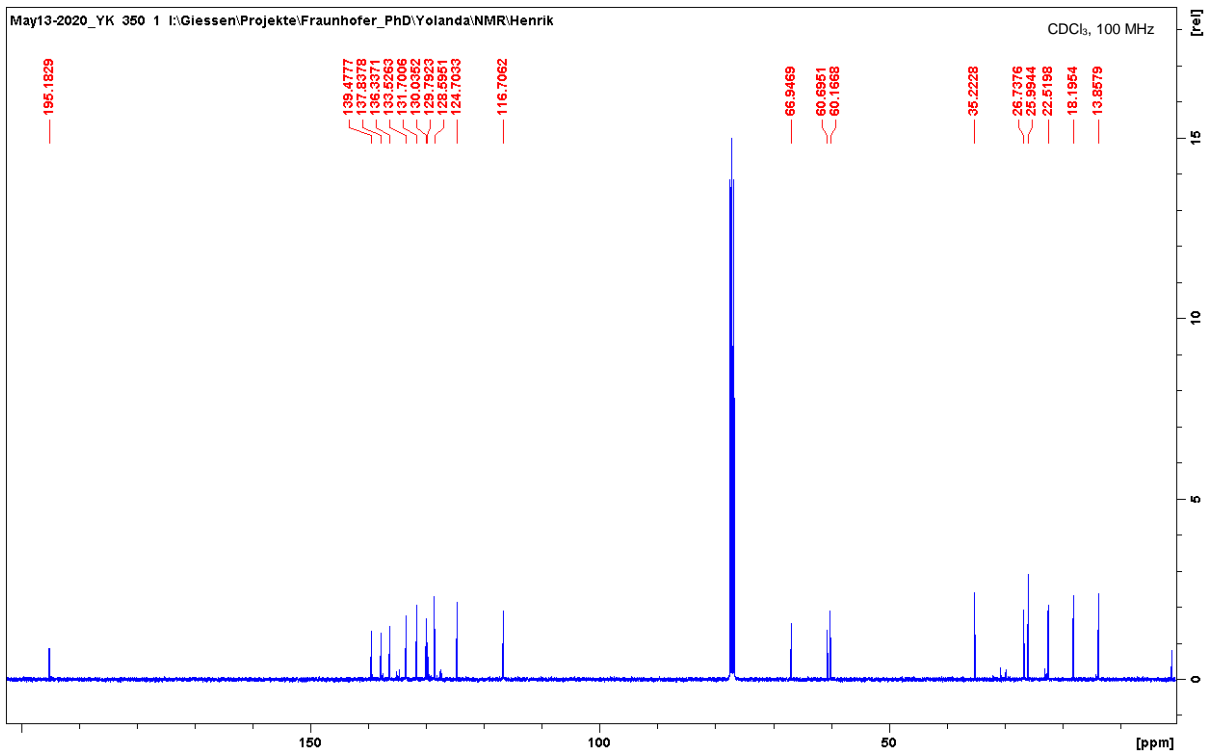
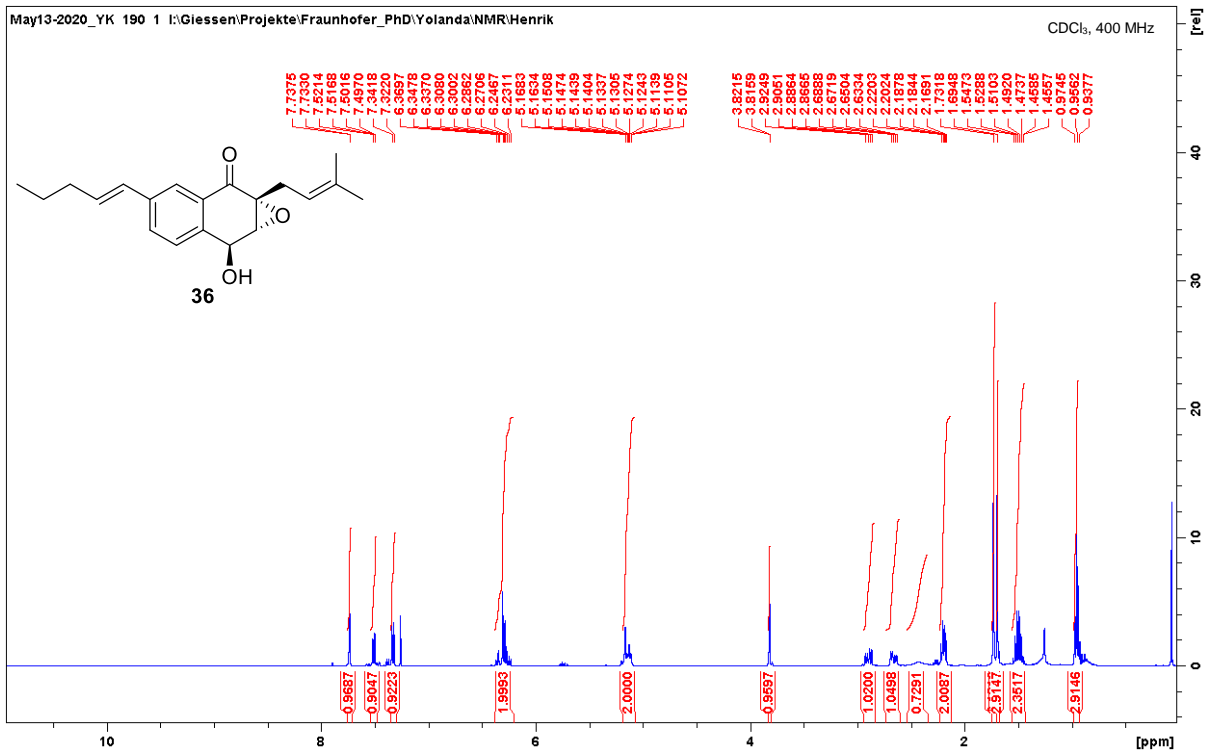










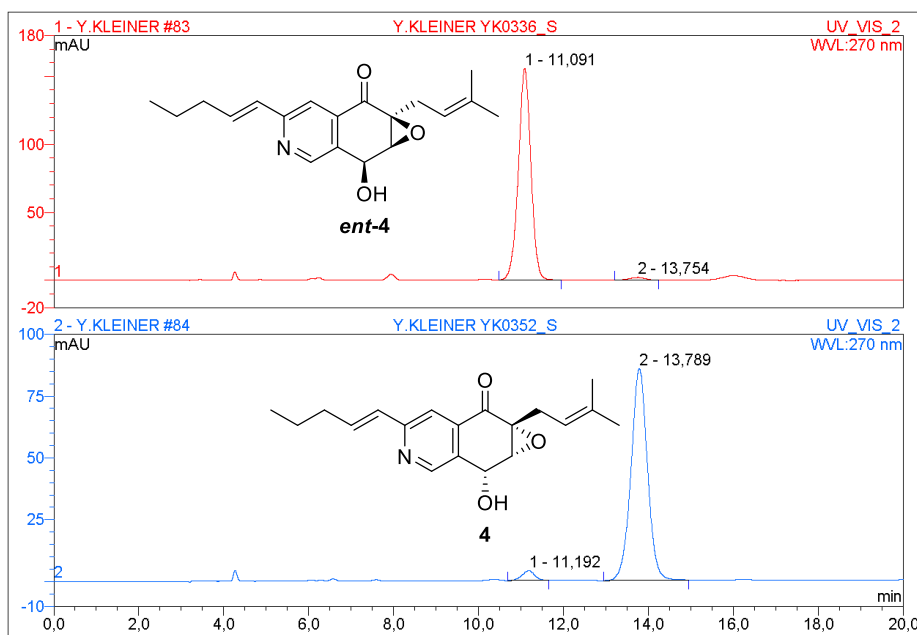


6. Determination of the enantiomeric excesses (ee)

Operator:ge1585 Timebase:HPLC_1 Sequence:Y.KLEINER

Page 1-1
19.5.2020 1:30 PM

84 Y.KLEINER YK0352_S			
CHIRALPAK IC 1ml/min. 85% Hexan, 15% IPA			
Sample Name:	Y.KLEINER YK0352_S	Injection Volume:	5,0
Vial Number:	85	Channel:	UV_VIS_2
Sample Type:	unknown	Wavelength:	270
Control Program:	Analyse03_F10_A85_B00_C15	Bandwidth:	1
Quantif. Method:	default	Dilution Factor:	1,0000
Recording Time:	19.5.2020 12:44	Sample Weight:	1,0000
Run Time (min):	20,00	Sample Amount:	1,0000

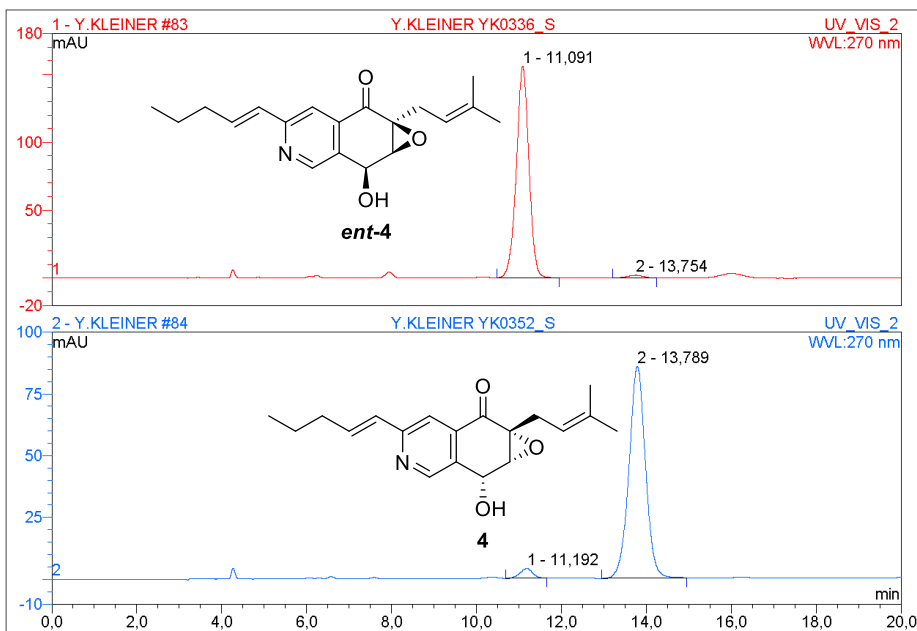


No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	11,19	n.a.	3,865	1,334	3,35	n.a.	BMB
2	13,79	n.a.	85,720	38,537	96,65	n.a.	BMB
Total:			89,585	39,872	100,00	0,000	

default/Integration

Chromeleon (c) Dionex 1996-2001
Version 6.80 SR15 Build 4656 (243203)

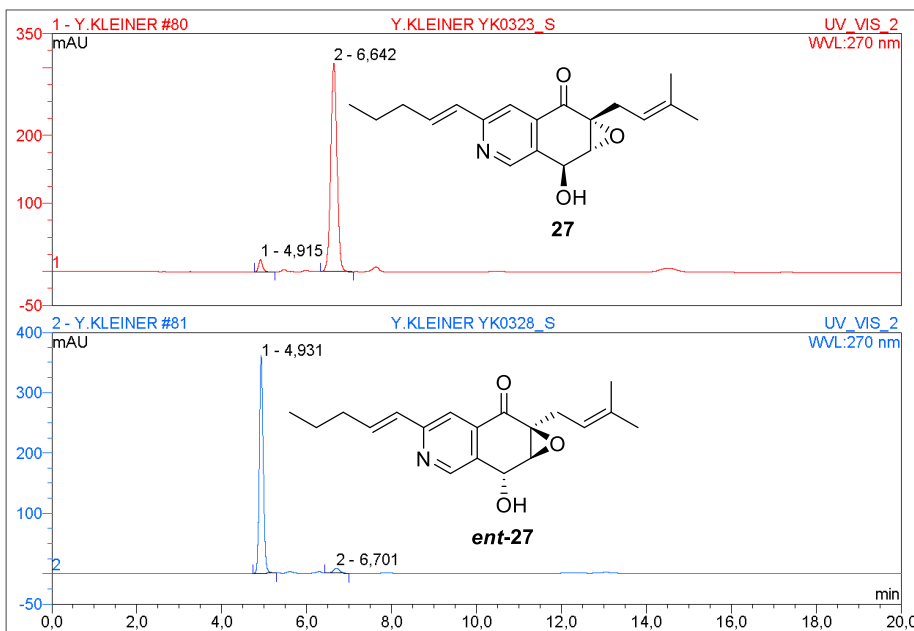
83 Y.KLEINER YK0336_S		
CHIRALPAK IC 1ml/min. 85% Hexan, 15% IPA		
Sample Name:	Y.KLEINER YK0336_S	Injection Volume: 5,0
Vial Number:	84	Channel: UV_VIS_2
Sample Type:	unknown	Wavelength: 270
Control Program:	Analyse03_F10_A85_B00_C15	Bandwidth: 1
Quantif. Method:	default	Dilution Factor: 1,0000
Recording Time:	19.5.2020 12:17	Sample Weight: 1,0000
Run Time (min):	20,00	Sample Amount: 1,0000



No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	11,09	n.a.	155,502	53,258	98,54	n.a.	BMB
2	13,75	n.a.	1,900	0,787	1,46	n.a.	BMB
Total:			157,402	54,045	100,00	0,000	

80 Y.KLEINER YK0323_S**CHIRALPAK IC | 1ml/min. | 85% Hexan, 15% IPA**

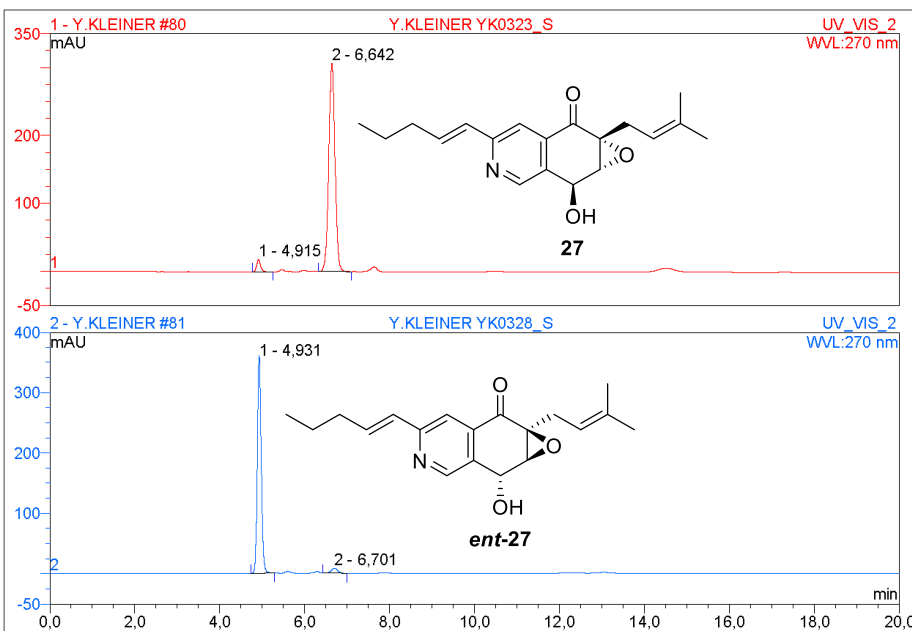
Sample Name:	Y.KLEINER YK0323_S	Injection Volume:	2,5
Vial Number:	81	Channel:	UV_VIS_2
Sample Type:	unknown	Wavelength:	270
Control Program:	Analyse03_F10_A85_B00_C15	Bandwidth:	1
Quantif. Method:	default	Dilution Factor:	1,0000
Recording Time:	18.5.2020 9:40	Sample Weight:	1,0000
Run Time (min):	20,00	Sample Amount:	1,0000



No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	4,92	n.a.	18,151	1,921	3,55	n.a.	BMB
2	6,64	n.a.	306,543	52,173	96,45	n.a.	BMB
Total:			324,694	54,095	100,00	0,000	

81 Y.KLEINER YK0328_S**CHIRALPAK IC | 1ml/min. | 85% Hexan, 15% IPA**

Sample Name:	Y.KLEINER YK0328_S	Injection Volume:	2,5
Vial Number:	82	Channel:	UV_VIS_2
Sample Type:	unknown	Wavelength:	270
Control Program:	Analyse03_F10_A85_B00_C15	Bandwidth:	1
Quantif. Method:	default	Dilution Factor:	1,0000
Recording Time:	18.5.2020 10:26	Sample Weight:	1,0000
Run Time (min):	20,00	Sample Amount:	1,0000



No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	4,93	n.a.	360,673	38,359	96,46	n.a.	BMB
2	6,70	n.a.	8,238	1,409	3,54	n.a.	BMB
Total:			368,912	39,768	100,00	0,000	